The Journal of HEREDITY

UNCLOVEN HOOFS
(SEE PAGE 241)

Hybridization in Tapeworms

Roan and "Whitish" Guinea Pigs

A Non-Dominant Glaucoma Pedigree

Mass Selection of Blight-Resistant Oats

The Ultrasonic-Susceptible X-Chromosome

VOLUME 42 SEPTEMBER-OCTOBER, 1951 NUMBER 5

The American Genetic Association

An incorporated organization devoted to promoting a knowledge of the laws of heredity and their application to the improvement of plants, animals, and human racial stocks

Honorary Emeritus President, DAVID FAIRCHILD

MARCUS RHOADES, President — Vice-President, HUGH C. McPHEE

Honorary Vice-President, W. E. CASTLE

RONALD BAMFORD, Secretary — Treasurer, SAMUEL L. EMSWELLER

ROBERT C. COOK, Managing Editor — Assistant Editor, BARBARA LAKE

EDITORIAL BOARD

M. DEMEREC — RALPH W. PHILLIPS

H. J. MULLER — LAURENCE H. SNYDER — PAUL POPENOE

Members of the Council are ex-officio members of the Editorial Board

COUNCIL

RONALD BAMFORD, Dean of the Graduate School, University of Maryland, College Park, Md.

GEORGE M. DARROW, Horticulturist in Charge of Small-Fruit Breeding, Horticultural Field Station, Beltsville, Maryland.

HAIG DERMEN, Cytologist, Division of Fruit and Vegetable Crops and Diseases, Plant Industry Station, Beltsville, Maryland.

SAMUEL L. EMSWELLER, Principal Horticulturist, Division of Fruit and Vegetable Crops and Diseases, Plant Industry Station, Beltsville, Md.

DAVID FAIRCHILD, Agricultural Explorer (Retired), U. S. Department of Agriculture.

WALTER E. HESTON, Geneticist, National Cancer Institute, U. S. Public Health Service, Bethesda, Md.

H. J. MULLER, Professor of Zoology, Indiana University, Bloomington, Indiana.

HUGH C. McPHEE, Assistant Chief of Animal Husbandry Division, U. S. Department of Agriculture, Washington, D. C.

MARCUS M. RHOADES, Professor of Botany, University of Illinois, Urbana, Illinois.

LAURENCE H. SNYDER, Dean of the Graduate School, University of Oklahoma, Norman, Oklahoma.

WILLIAM VOGT, Author and Conservationist, Williamsburg, Virginia.

SEWALL WRIGHT, Professor of Zoology, University of Chicago, Chicago, Illinois.

ADVISORY COMMITTEE

E. B. BABCOCK, Genetics Department, University of California, Berkeley, California.

E. A. BESSEY, Professor of Botany, Michigan State College, Lansing, Michigan.

A. F. BLAKESLEE, Professor of Botany, Smith College, Northampton, Mass.

W. E. BRYAN, Professor of Plant Breeding, University of Arizona, Tucson, Arizona.

RUSSELL W. DUCK, Editor, *The Rural New Yorker*. New York City.

C. M. GOETHE, Sacramento, California.

HARRISON R. HUNT, Professor of Zoology, Michigan State College, East Lansing.

D. F. JONES, Plant Breeder, Connecticut Agricultural Experiment Station, New Haven, Conn.

M. A. JULL, Professor of Poultry Husbandry, University of Maryland, College Park, Md.

C. C. LITTLE, Director, Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine.

JAY L. LUSH, Professor in Animal Husbandry, Iowa State College, Ames, Iowa.

H. H. NEWMAN, Emeritus Professor of Zoology, University of Chicago, (Clearwater, Fla.).

T. S. PALMER, Expert in Game Conservation, U. S. Dept. of Agriculture, Washington, D. C.

JOHN H. PARKER, Director, Midwest Barley Improvement Association, Milwaukee, Wisconsin.

JOHN W. SCOTT, Professor of Zoology, University of Wyoming, Laramie, Wyoming.

A. D. SHAMEL, Physiologist, U. S. Department of Agriculture, Riverside, California.

GEORGE H. SHULL, Professor of Biology, Princeton University, Princeton, New Jersey.

EDMUND W. SINNOTT, Professor of Botany, Yale University, New Haven, Conn.

W. W. SMITH, Professor of Animal Husbandry, Purdue University, Lafayette, Indiana.

A. B. STOUT, Director of Laboratories, New York Botanical Gardens, New York City.

W. T. SWINGLE, Plant Physiologist (Retired), U. S. Department of Agriculture.

W. H. TOMHAVE, American Aberdeen-Angus Breeders' Association, Chicago, Ill.

E. N. WENTWORTH, Armours' Bureau of Agr'l Research and Economics, Chicago, Illinois.

P. W. WHITING, Zoological Laboratory, University of Pennsylvania, Philadelphia, Pa.

The JOURNAL OF HEREDITY is published bi-monthly by the AMERICAN GENETIC ASSOCIATION at 32nd Street and Elm Avenue, Baltimore 11, Md., and 1507 M St., N. W., Washington 5, D. C. Address all communications regarding editorial matters to 1507 M St., N. W., Washington, D. C. Communications regarding the business management of THE JOURNAL OF HEREDITY may be addressed to the publication office, 32nd Street and Elm Avenue, Baltimore, Md., or to 1507 M St., N. W., Washington 5, D. C. Entered as second-class matter February 24, 1923, at the postoffice at Baltimore, Maryland. Acceptance for mailing at the special rate of postage provided for in section 1103, Act of October 3, 1917, authorized December 29, 1918.

Contents copyrighted 1951 by the American Genetic Association. Unauthorized reproduction of material from THE JOURNAL not permitted. The title "Journal of Heredity" is registered as a trade-mark in the U. S. Patent Office.

$5.00 a Year. $1.00 a Copy. Prices of back numbers on application.

The

Journal of Heredity

Vol. XLII **SEPTEMBER-OCTOBER, 1951** **No. 5**

CONTENTS

THE PHYSICO-CHEMICAL PROPERTIES OF THE SEX CHROMOSOME
By G. Yasuzumi and A. Kondo 219

MASS SCREENING IN OAT BREEDING
By S. S. Ivanoff 225

WHITISH, A MODIFIER OF CHOCOLATE AND BLACK HAIRS IN GUINEA PIGS
By Heman L. Ibsen and Betty Lanning Goertzen 231

MULLER'S METHOD OF CALCULATING POPULATION SIZES
By G. B. Mainland 237

SYNDACTYLISM IN HOLSTEIN-FRIESIAN CATTLE
By F. E. Eldridge, W. H. Smith and W. M. McLeod 241

CYTOLOGICAL EFFECTS OF SOME MORE ANTIBIOTICS
By G. B. Wilson and C. C. Bowen 251

STUDIES ON SEMI-LETHALISM IN THE MALE LORDOTIC GUPPY
By Harold L. Rosenthal 257

RACIAL DIFFERENCES IN PALM-PRINT RIDGE COUNTS
By T. C. Fang 261

HYBRIDIZATION IN THE TAPEWORM
By Arthur W. Jones 264

ROAN, A MODIFIER OF PIGMENTED HAIRS IN GUINEA PIGS
By Heman L. Ibsen and Betty Lanning Goertzen 267

A NOT DOMINANT GLAUCOMA PEDIGREE
By Richard B. Goldschmidt 271

OBSERVATIONS ON MIDGETS IN BEEF CATTLE
By C. E. Lindley 273

Notes and Reviews

J'ACCUSE
By Frederick S. Hammett 223

THE GROWING BACKLOG OF GENETIC DEFECT
By H. J. Muller 230

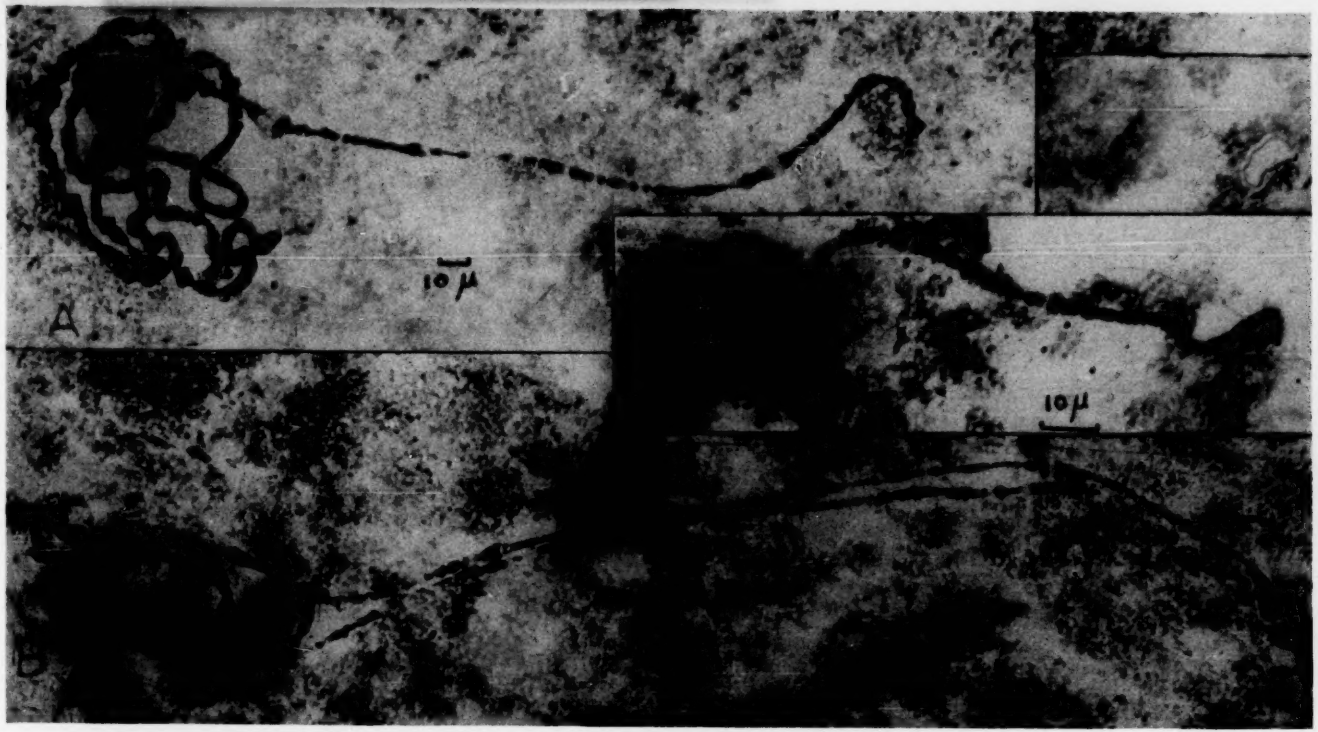
THREE IN ONE: PHYSICAL ANTHROPOLOGY, GENETICS, STATISTICS
(Rev. of Boyd: *Genetics and the Races of Man*)
By T. D. Stewart 255

A SURGICAL TECHNIQUE FOR THE RECIPROCAL TRANSPLANTATION OF FERTILIZED EGGS
By Frederick R. Avis and Paul B. Sawin 259

PROGENY TESTS AND WARWICK'S TABLES
By F. B. Hutt 266

THE EFFECT OF ALTITUDE ON GERM CELLS IN RELATION TO CYTOLOGICAL TECHNIQUE
By J. C. Cross 269

THE PROBLEM OF EPILEPSY
(Rev. of Alstrom: *A Study of Epilepsy in its Clinical, Social and Genetic Aspects*)
By B. Ascher and F. J. Kallmann 275



SEX CHROMOSOMES "STRETCHED" BY ULTRASONIC VIBRATIONS

Figure 1

These three photographs show the response in two species of *Drosophila* to exposure for one minute to ultrasonic vibration having a frequency of 450,000 vibrations per second. *A* and *B* show preparations of *Drosophila virilis*. In *B* the fourth chromosome has also responded to the ultrasonic vibration, which happens occasionally in the preparations observed. *C* shows a preparation of salivary gland chromosomes of *Drosophila melanogaster*, with the X-chromosome affected. Occasionally in this species the third chromosome also responds. *A* and *B* are enlarged about 230×; *C* is enlarged about 640×.

THE PHYSICO-CHEMICAL PROPERTIES OF THE SEX CHROMOSOME

A Study By Means of Ultrasonic Waves

G. YASUZUMI AND A. KONDO

*Anatomical Department, School of Medicine, Osaka University, Osaka, Japan**

THE discovery of the sex chromosomes was an important landmark in the history of genetics.[1] In 1902 McClung[3] first discovered chromosomes which formed an unequal pair in one sex, while corresponding to them in the other sex was an equal pair. These chromosomes therefore behave like the postulated sex factors and were called the sex chromosomes, the remaining chromosomes being called the autosomes. Specialized sex chromosomes have since been found in a great many animals and plants. They are easily identified according to their size, shape and heterozygosity in the male or female in many species of the animal and vegetable kingdoms. The difference between sex chromosomes and autosomes has never been chemically analyzed as they are too small to lend themselves to analysis by existing methods.

The authors have attempted by a number of methods to demonstrate a difference in physico-chemical properties between sex chromosomes and autosomes. These have included comparisons of pH, staining in male and female hormone fluids, argentophy, various amino acids-, protein-, lipids-, glycogen-, and enzyme-reactions. All these experiments were negative. We are now fortunate in being able to report that we have succeeded in identifying a notable difference in physico-chemical reaction between the sex chromosomes and autosomes in Dipteran salivary glands exposed to ultrasonic vibration.

Material and Method

All the experiments to be described were carried out on wild-type flies of *Drosophila melanogaster* and *virilis* cultured in our laboratory. Full-grown larvae, ranging from seven to eight mm in length, were employed. The apparatus consisted essentially of a quartz crystal operated at 2,000 volts, 210 milliampere and with a frequency of 450,000 in an oil bath.

The vibration of the crystal is transmitted to the oil and to a glass tube immersed in the oil. In the experiments the larvae were placed in a test tube. The difference could not be demonstrated when excised salivary glands were exposed directly to ultrasonic waves. When this was attempted cells and nuclei were destroyed so suddenly that it was impossible to investigate the structure of the chromosomes.

The larvae were placed in the test tube on cotton which had been dipped in insect Ringer's solution. The tube containing the larvae was put on the surface of the oil. After an exposure of one minute, the glands of larvae were dissected out in insect Ringer's solution. The gland was transferred into a drop of fixative-stain containing 45 percent acetic acid and saturated carmin. After one minute at 18° C, a clear cover slip was mounted over the preparation, and the cells were then compressed with a mercury filled bottle rolled over the cover slip. The preparations were then ready for examination under the light microscope.

In order to make clear the finer structure of salivary chromosomes subjected to ultrasonic waves, replicas of the treated chromosomes were photographed under the electron microscope. The method used was as follows: The gland was crushed in a drop of 45 percent acetic acid without carmin on a glass slide. After adequate drying the cover slip was immersed in absolute alcohol. It was then easily removed from the slide, leaving some of the chromosomes attached to the slide. For further fixation and progressive dehydration the crushed preparations were immersed in 95 percent ethyl alcohol for at least 24 hours. The preparations were placed in a desiccator over phosphorous pentoxide until all visible moisture had vanished and they were finally heated over a heater for a moment. Replicas were obtained by immersing glass slides supporting the dried specimen in 0.75 percent solution of formvar in ethylene dichloride and al-

*This work was supported in part by grant from the Scientific Research Council of Japan. We wish to express our sincere thanks to Prof. T. Tomita, Prof. T. Komai, Prof. H. Oyama, Prof. T. Kasai and Mr. A. Tanaka for their valuable advice and help in the course of this work.

FINE STRUCTURE OF TREATED CHROMOSOMES

Figure 2

This electron micrograph (5000×) of a formvar replica of a stretched sex chromosome of *Drosophila virilis*, shows the destruction of the beaded structure of the chromatin matter. Only when living larvae were exposed to the high frequency vibrations was the chromosome structure retained even to this extent. Dissected preparations of salivary gland chromosomes exposed to the vibration for one minute were completely destroyed.

lowing the preparation to dry in air while in the vertical position. The film was stripped off the slide in water, and moved over a sample holder which had been already placed alongside. The hole in this holder was 0.1 mm in diameter. The preparation was drained on blotting paper and allowed to dry in air. The exposed side of the replicas were then shadowed with chromium in the usual manner. Most of the work was done at a shadowing angle of 25°.

Results

Most of the experiments have been attempted with female larvae. We shall describe the results of study with the reproductions of light and electron micrographs. In the first series of experiments we have observed the effect of chromosomes subjected to the ultrasonic waves by the help of a light microscope. Figure 1*A* shows a micrograph of the salivary gland chromosomes of *Drosophila virilis* subjected to the treatment with ultrasonic waves. A well defined nucleolus and five chromosomes surrounding it are clearly visible, while the sex chromosome appears in remarkably stretched condition. When larvae have been treated with ultrasonic waves for less than one minute, we have never failed to obtain this figure.

Very rarely the fourth chromosome is revealed running parallel with the sex chromosome, as shown in Figure 1*B*. The figures illustrate the fact that some interchromatin regions are expanded in the spindle shape, which has never been seen in the normal stretched salivary chromosomes. The interchromatin regions appear to be stretched, while chromatin regions remain unstretched.

In the salivary gland chromosomes of *Drosophila melanogaster*, the effect of ultrasonic waves on the third chromosome has occasionally been seen, stretching together with the sex chromosome in this species. When male larvae were exposed to ultrasonic waves for one minute, the sex chromosome has been remarkably stretched, because in the male this represents a single chromosome and not a fused pair as are the autosomes.

In a second series of experiments we have observed with the electron microscope the microstructure of the sex chromosome subjected to ultrasonic waves. It has been already confirmed[9] that the interchromatin regions are made up of longitudinal strands which consist of a beaded structure with a periodicity that has averaged between ca. 30mμ and 40mμ. As is shown in Figure 2, we have failed to observe such a beaded structure in the treated chromosome because of the destructive effect of ultrasonic waves upon living organism. The particles of ca. 100mμ in diameter have been distributed in an irregular pattern between the chromonemata, though in some cases the particles are aligned along the length of the chromonemata in a very regular pattern. The diameter of a chromonema-bundle is probably more than 100 mμ.

In a few cases, the separation of chromonemata-bundles in the sex chromosome has been encountered (Figure 3). The single thread is about 20mμ wide. It is difficult to separate the chromonemata-bundle. Only ultrasonic vibration has enabled us to do this.

Figure 4 demonstrates the microstructure of interchromatin region expanded in a spindle shape. Most of the chromonemata-bundles have been destroyed, so that we cannot trace the chromonemata. They look like a chromatin region. Since this expanded region gives a negative Feulgen reaction when tested after the replicas are made this may be taken as evidence that it is nothing but an interchromatin region.

Discussion

Since the first description by Wood and Loomis,[5] of phenomena connected

CHROMONEMA-BUNDLES SEPARATED

Figure 3

Electron micrographs of the formvar replica of salivary gland sex chromosome of *Drosophila virilis* treated with ultrasonic vibration for one minute. The chromonemata-bundles, of which the salivary gland chromosomes are believed to be built up, have been separated by the action of the ultrasonic vibration.

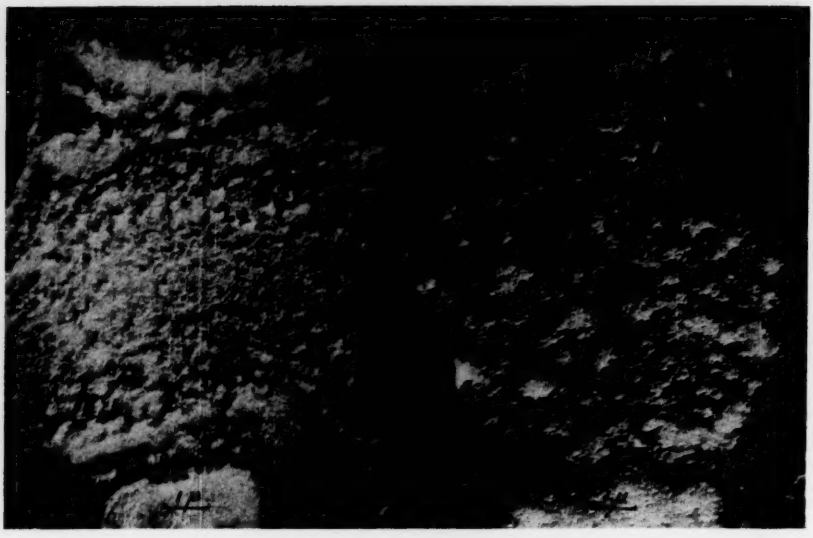
with high frequency and high intensity sound waves, a number of workers have investigated more fully the physical, chemical and biological effects of these ultrasonic vibrations. Thus, Yasuzumi and co-workers[10,11] have observed mechanical and oxidation effects of ultrasonic waves on the salivary chromosomes.

Exposure to ultrasonic waves with a frequency of 450,000 for one minute seems to have a specific influence on the sex chromosomes. The authors regret that they have not been able to observe the effects of ultrasonic waves of other frequencies on the salivary chromosomes.

The electric field and the ultrasonic waves heated the oil and Ringer's solution in the test apparatus. Exposure for one minute raised the temperature of 5cc of Ringer's solution in the test tube by 5° C (the room temperature was 18° C). Presumably none of the effects reported are due to temperature change.

The extensibility of the sex chromosomes seems to differ from that of the autosomes, the latter being much more rigid. Two factors may account for this difference. Firstly, if it is assumed that autosomes are formed from β-polypetide chains and the sex chromosomes are constructed from α-polypeptide chains, the latter probably can be stretched farther than the former when an effective stretching technique is used. Secondly, the sex chromosomes may be composed of globular proteins which have a more resonant response to a 450 kilocycle vibration than the autosomes. Much further study is required, however, before these can be taken as more than suggestions.

The chemical composition of the respective chromosomes is entirely unknown. It has been, however, currently confirmed that the constituents of salivary chromosomes are desoxyribonucleic acids and protein molecules which contain arginine, glycine, alanine, valine, leucine, serine, aspartic acid, glutamic acid and proline.[7] The salivary chromosomes include histone which has a high content of arginine.[8] The clupein mole-



DAMAGE TO CHROMONEMATA

Figure 4

These are electron replica-micrographs of sex chromosomes in the salivary gland of *Drosophila virilis*. The microstructure of interchromatin regions have expanded in a spindle shape. Most of the chromonemata-bundles have been destroyed, so that we cannot trace the chromonemata. 7000×.

cule is said to reach a maximum length of 98 Å, being made up of 28 residues of which 20 are arginine.[2] However, the visible chromonema is 20mμ thick in the salivary chromosomes. This value supports the findings of Yasuzumi[6] in the unit of the salivary chromosomes separated in cold water.

Palay and Claude[4] and Yasuzumi, Odate and Ota[9] have stated that nucleic acid molecules seem to lie on the surface of each chromomere. The nucleic acid molecules are frequently coalesced to form the discs. The proteins in the chromatin discs have a particular affinity for nucleic acid and nucleic acid is in a highly polymerized form. Any vibration does not destroy the chromatin discs so easily. The nucleic acid has presumably a function of stabilizing the gene locus. The chromonema may be comparable to a violin string, and chromatin disc to the bridge of that instrument.

The fourth chromosome in *Drosophila virilis* and the left arm of the third chromosome in *Drosophila melanogaster* sometimes, though very rarely, are found stretched out remarkably along with the sex chromosome, under the influence of the ultrasonic waves. The sex chromosome, in its course of running, may have accompanied these autosomes which had been attached to it towards its own way.

Summary

It has been attempted to identify the difference of physico-chemical property between the sex chromosome and autosomes with the help of ultrasonic waves. When the female larvae of *Drosophila virilis* or *melanogaster* have been exposed to the ultrasonic waves with 450 kilocycles for one minute, the sex chromosome has been remarkably stretched. The interchromatin regions have been expanded in a spindle shape, which shows that most of the chromonemata have been destroyed. In some interchromatin regions the separation of a chromonemata-bundle is observed, the diam-

eter of each chromonema being 20mμ. And in others the chromonemata are found destroyed giving no trace of the beaded structure.

Literature Cited

1. For a history of the impact of the sex chromosomes on the development of genetics and cytology see STURTEVANT, A. J., in Genetics in the Twentieth Century, L. C. Dunn, Editor, Macmillan Co., New York, 1951.
2. LINDERSTRØM LANG, K., and K. E. RASMUSSEN. *Comp. Rend. Lab. Carlsberg.* 20:1. 1935.
3. MCCLUNG, C. E. *Biol. Bull.* 9:75. 1902.
4. PALAY, S. L., and A. CLAUDE. *J. Exp. Med.* 89:431. 1949.
5. WOOD, R. W., and A. L. LOOMIS. *Phil. Mag.* 4:417. 1927.
6. YASUZUMI, G. *Cytologia* 14:1. 1947.
7. ———— and G. MIYAO. *Exp. Cell Research 2*: 153. 1951.
8. ————, T. MORI, H. MATUKURA, and R. MINAMINO. *Cytologia* 15:173. 1950.
9. ————, Z. ODATE, and Y. OTA. *Cytologia.* 1951. (in press).
10. ————, and Y. YOSHIDA. *Cytologia* 15:255. 1950.
11. ————, Y. YOSHIDA, and S. NANJO. *Cytologia* 15:299. 1950.

(*Received May 1, 1951*)

J'ACCUSE!

Federal Science and Thought Control

IT apparently is not yet too late to erase from the books that attempt at socialization of science in the U.S.A. yeclept The National Science Foundation.

Every young worker in science is aware of the subtle pressure exerted on him to make his research, his conclusions, and his ideas conform to those of his superior. Under pain of not getting published. Or of even losing his job. And every mature, competent, independent-minded creative worker knows the fight he has had to wage to get his work and ideas considered: if he has at any time evidenced independence of spirit and concepts.

One example: the man who made the most outstanding forward step in cancer research in our time was forced to leave the institution where he made his discovery and go elsewhere because as the Herr Direktor said: "He is hard to get along with." Thus the practical potentiality of the finding has been smothered and delayed to application for more than a decade.

Another example: it is essentially impossible to get into print any objection to this attempt at socialization of science under the alias of a National Science Foundation. The Big Shots brook no oposition. Through control of our media of expression.

To what depths of pusillanimous ignominy have our scientists fallen that they allow themselves to be led like the donkey with the carrot. Instead of rising up in loud objection: as has the medical profession against the socialization of their profession. Would that we had like men of guts and courage. Let the chips fall where they may.

The National Science Foundation should be wiped from the books. First because it is an attempt at grab for power by the High Brass in science. Who as every science worker knows demand obsequious subservience before granting approval of project or funds. And who, if such bowing of the knee is not given, say the project is impractical and the worker unsound. Boy, do I know 'em!

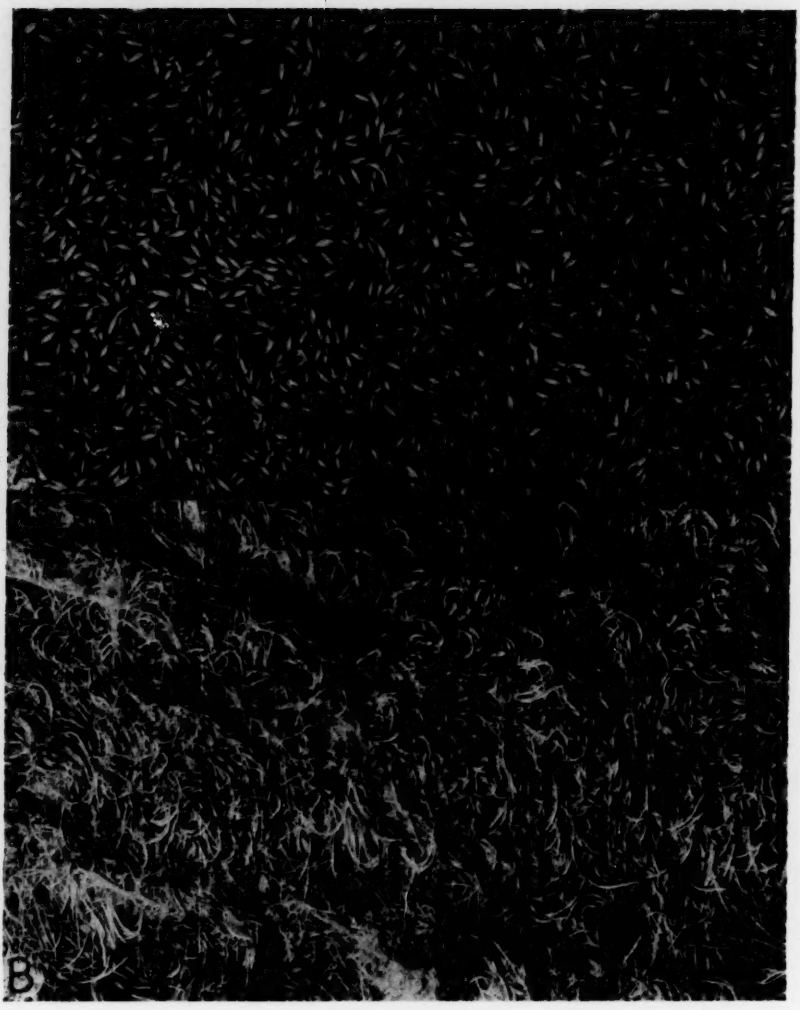
Second, because it is the entering wedge of thought control in science. Which destroyed creative leadership in science in Germany; is destroying such in Russia. But few of the readers of this accusation have not sensed or experienced such.

And third, because no independent creative thinker in science can or will submit to such restrictions upon his explorations into the unknown. Which means creative work and leadership will be strangled for years to come.

The remedy? Write your Congressmen and Senators. Show them that the scientific as well as the medical profession is well aware of the implications of a National Science Foundation. Will have none of it. You may lose your job. You may not get funds for your work. But what profit it a man if he gain the world but lose his soul? By the way —how many of you had the chance to express your opinion on a National Science Foundation in *secret* ballot? I didn't. Did you?

Young and old workers in science who have visited me in this remote and essentially inaccessible retreat have expressed like opinions. But the beliefs herein stated are mine own and not necessarily those of the Institution with which I am connected. Its opinions I do not know. Thus it (and THE JOURNAL OF HEREDITY) is hereby absolved from all responsibility therefor.

FREDERICK S. HAMMETT
Provincetown, Mass.



MASS SCREENING OF VICTORIA-BLIGHT SUSCEPTIBLE OATS

Figure 5

A—Part of a mass of approximately 7500 oat seeds of susceptible variety treated in one "rag-doll" and kept at 28° C. for seven days. *B*—A comparable sample of seeds of a resistant variety following the same treatment.

MASS SCREENING IN OAT BREEDING

A Seed Laboratory Method for Testing Resistance of Oats to Victoria Blight*

S. S. IVANOFF

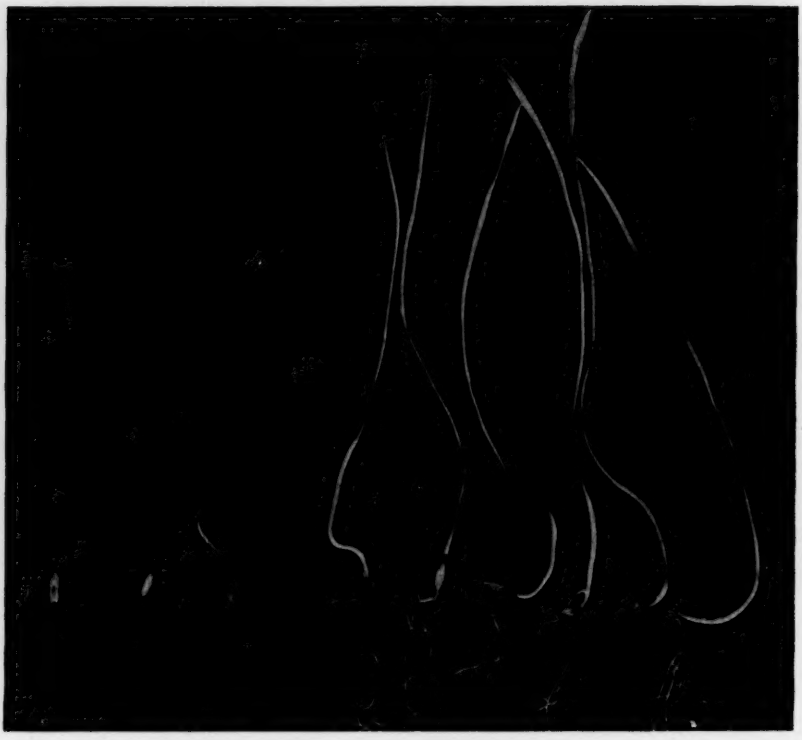
THE efforts of oat breeders have recently been directed toward the development of new varieties which display resistance to the two most important oat diseases, crown rust (*Puccinia coronata* (Pers.) Cda), particularly races 45 and 57, and to Victoria blight (*Helminthosporium victoriae* Meehan and Murphy). For most locations in the South, winter-hardiness and capacity for producing abundant green forage for grazing are also considered in the breeding program. Such oat strains are now being developed by cross breeding, utilizing as sources of resistance, certain native oats and the foreign varieties Santa Fe, Trispernia, Landhoffer, Ukraine and some others. Another possible approach toward the acquirement of such combined resistance may be attempted through an intensive search, within large populations of the Victoria derivatives, for certain rare segregates which might exist apart from the linkage relationship between susceptibility to Victoria blight, and resistance to crown rust, "Victoria type."† [6,9] Regardless of the kind of approach adapted, any improvement in the techniques for testing disease resistance would be of help in speeding up this complex breeding program.

In the past various methods of testing resistance to Victoria blight have been used. [1,8,13] Plant breeders have attempted artificial inoculations using small plants in the greenhouse with different results: by spraying or by rubbing the leaves with a culture of the pathogen, by puncturing, use of ground infected straw, macerated cultures with and without agar, etc. Others depend on naturally or artificially infested plots where segregating populations are grown and the susceptible individuals eliminated during the growing season. The susceptibility of an oat variety has also been determined quickly by germinating seeds in petri dishes where seedlings of a susceptible variety deteriorated very rapidly.[13] With some greenhouse methods incubation of inoculated plants in moist chambers was necessary for complete elimination of susceptible plants. Emergence of susceptible varieties in the greenhouse varied from 0 to 100 percent, depending upon the method of inoculation.[8]

What is necessary perhaps, in connection with this and many other similar problems in breeding for disease resistance is a new departure from the more conventional greenhouse and field methods of testing resistance. There is a need for a mass process for screening out hundreds of thousands of susceptible individuals quickly and efficiently in the pre-seedling stage, a process to embody to the greatest possible measure the combined concepts of mass, speed, and exactitude. A simple procedure may be envisaged by which the testing might be done in the laboratory with

*From the Department of Plant Pathology and Physiology, Mississippi Agricultural Experiment Station, State College, Mississippi. Journal Paper (New Series) No. 255.

† This is the name given to a hypersensitive type of resistance, or specifically to the local reaction of the host tissue towards the ingress of the rust parasite, presumably resulting from the severing of the food supply through collapse of cells surrounding the point of infection. This type of resistance is exhibited by a characteristic spotting of the leaves in varying degrees by most, but not all, varieties originating from a Victoria cross.



SUSCEPTIBLE VS. RESISTANT OATS

Figure 6

At the left are six representative seeds of Victoria-blight susceptible oats from a "rag-doll" tested by this method. Note that roots are entirely absent or that only disintegrating vestiges remain. At the right are six oat seedlings resistant to this blight which have undergone the same treatment.

relatively large and compact masses of seeds, in place of potted plants or other conventionally grown plants in the greenhouse or in the field, and the results made known in a few days before much growth, if any, had developed. It was thought that, under certain conditions yet to be determined, seeds carrying in their germ plasm susceptibility to the disease might react to the presence of the parasite and its toxins in the same definite manner as seedlings or more mature plants, perhaps even more decisively so. Similar concepts were previously followed in an attempt to develop a laboratory seed method for testing cold hardiness in oats.[4,5]

The present report gives an account of the development and the experimental performance of such a laboratory method for processing large masses of seeds in selecting for disease resistance.

The Method

Essential factors. This method consists simply in coating oat seeds with an agar culture of the causal organism in a definite concentration, placing the coated seeds between wet paper towels, sacks, or rags, rolled in a manner to form "rag-dolls," and maintaining same at 25° to 30° C. In five to seven days the test is completed. The susceptible seeds either do not germinate or if they do germinate their roots become necrotic and quickly disintegrate,

while the resistant seeds germinate and develop as more or less normal seedlings. If left in the "rag-doll" for a longer time many of the susceptible seeds develop slender coleoptiles, but no roots (Figure 6).

Before arriving at this simple procedure, however, many failures were encountered because of inadequate understanding of certain essential factors which, working together, inhibit or kill seeds of susceptible varieties. These factors are the following:

1. It seems necessary for the fungus and its accumulated toxins to be present in a nutrient colloidal vehicle, such as an agar medium, in a definite high concentration. Other colloidal substances such as corn starch and flour were tried with equal success, but agar was found the most convenient. On the other hand, soaking the seeds in liquid culture media of different ages and for various lengths of time, but in the absence of the necessary amount of colloidal material, did not produce the results sought. Such procedures delayed or prolonged the destructive process, or resulted in many escapes from the disease. There is some evidence that the agar medium itself, without the fungus and the toxins, exerts a predisposing influence on the seed. The agar medium may also produce a slight stunting effect on some resistant seedlings, but no discoloration, decay or death.

2. It is required that the treated seeds are kept in an environment of 100 percent relative humidity, and with a slight amount of free moisture. Any departure from this condition results in failure to kill, in undesirable prolongation of the root-rotting process, or in escapes. This essential point is easily met, however, by the conditions present in a wet "rag-doll."

3. The temperature at which the inoculated seeds are kept is equally important. Fortunately the desired range is relatively wide and commonly available. The optimum temperature appears to be near 28° C., but quite satisfactory results were obtained anywhere between 25° and 30° C. Lower temperatures seemed to diminish the effects of this fungus and its toxins without placing the germinating seed at a noticeable disadvantage.

4. The inclusion of common fungus foods and nutrients in the colloidal material is important, particularly the presence of available carbohydrates. These are placed originally in the agar medium, or if exhausted by an old culture, are added to the agar before treating the seed.

Practical Procedure for Testing Small Lots of Seed

The fungus may be cultured on any one of the commonly used bacteriological agar media, such as potato dextrose, nutrient dextrose, or vegetable agars with 15 gr. of dextrose and 17 grams of dry agar per liter of medium. The commercially prepared media are equally good and convenient. The cultures may be grown in test tubes, or if larger quantities are desired, on slants in 16-oz. medicine bottles.*

The agar culture is mixed with twice its volume of water in a common electric blender for 15 sec. When an old culture is used (20 to 30 day-old), one percent dextrose is added to the medium prior to blending. The seeds are placed in a small strainer, the agar mixture poured on, and the coated seeds spread on a wet paper towel. The seeds are covered by another wet towel and rolled into a "rag-doll." The roll is placed in a germinator at temperatures already stated, or if many "rag-dolls" are made, in an ordinary box or bell jar in a warm room. From a few to several hundred seeds may be placed in a "rag-doll" made of the average-size heavy paper towels, care being taken to insure that the tissue is kept wet constantly. Keeping the "rag-dolls" under a bell jar is very helpful because the rolls will thus need no further care. After the seventh day the disease resistant sprouts (with healthy white roots) may be picked out and transplanted if that is desired.

In preparing the culture mixture prior to treating the seed, the most important thing required is the proper dilution of the agar culture with water, namely, in the proportion of 1 part of the culture to 2 parts of water. Higher dilutions give disappointing results.

The above procedure is particularly convenient for selecting resistant individuals from the early segregating generations following a cross between a homozygous susceptible and homozygous resistant parent, or for determining in general resistance or susceptibility of homozygous varieties or strains of oats.

Testing Large Quantities of Seed

When a search is to be made for certain few resistant seeds that might exist in a large population of susceptibles, such as one in a thousand, or one in a hundred thousand, the following procedure may be followed: The seed is weighed out in 200 gr. lots, and each lot is placed in a one-quart-size ice cream container, or a similar vessel. Enough of the agar mixture, in the specified concentration, is poured into the container to fully cover all the seeds in it. A slight excess of the mixture is desirable. Approximately 200 cc. of the mixture is required to treat 200 gr. of seed. The seeds are then spread out on a wet gunny sack lined with wet paper towels and covered with wet paper towels and another sack, and

*Although slight differences in pathogenicity among different isolates of *H. victoriae* do exist, equally satisfactory end results were obtained with three different isolates, singly or in combination, including the standard culture, Iowa 224.

the two sacks rolled into a large roll in the fashion of a giant "rag-doll." Up to 50 seeds may be accommodated in one square inch area. When seeds of a homozygous susceptible variety have been used, they will all be dead or in a rotting condition five to seven days afterwards. If a single resistant seed is included among the many thousands of susceptible ones, such seed germinates normally, produces long, healthy-looking roots, and remains in a healthy condition indefinitely although appearing surrounded by the fungus growth and the many rotted seeds. On the other hand when an equally large lot of resistant seeds is treated and handled in the same way, the result is a matted growth of apparently healthy roots and coleoptiles, spread all over the gunny sack. (Figure 5).

Larger lots may also be employed with equal success and various other adaptations may be made for convenience, provided the essential factors are observed.

Testing the Method

At first, in the working out of the method, only two varieties of oats were used, one resistant and one susceptible. After the work was brought to a promising stage, a much larger number of both resistant and susceptible varieties of different origins were used to test further the efficiency and the reliability of the method. Of the susceptible varieties there were: Anderson, Arlington, Fulgrain, Letoria, Mustang, Stanton, Vicland, Victorgrain (2 strains), and Victoria, which is the oat introduced by the U. S. Department of Agriculture in 1927 from Uruguay, and from which the above varieties were derived by cross breeding with native oats.[2,3,14,15] Of the resistant varieties the following nine were tested: Bond, Bonda, Camellia, Carolina Red, Clinton, Delair, Delta Red 88, Forkedeer, and Nortex 107. The results of these tests demonstrated the high reliability of the method. In

TABLE I. Summary of the experimental performance of the seed laboratory method for testing resistance of oats to Victoria blight (*Helminthosporium victoriae*) with certain varieties known to be either susceptible or resistant.

No.	Varieties	C.I. number*	Origin	Germination of uninoculated seeds percent†	Inoculated Seeds: No. trials	Total No. replications	Total No. Seeds	Non-germinating (percent)	Healthy Seedlings (percent)
			A. Known Susceptible						
1	Anderson	4651	(Fulghum-Victoria) × Fulgrain (original C.I. 3253)	95	2	7	700	38	0.29
2	Arlington	4657	(Lee-Victoria) × Fulwin	99	2	5	500	12	0.00
3	Fulgrain	5356	[Norton 20-93 (Big Boy) × Navarro] × Victoria	94	2	7	700	34	0.00
4	Letoria	3392	Lee × Victoria	62	2	7	700	72	0.42
5	Mustang	4660	Fulwin × (Lee Victoria)	99	2	7	700	8	0.01
6	Stanton	5116	Lee × Victoria	98	2	7	700	11	0.42
7	Vicland	3611	Victoria × Richland	99	2	5	500	63	0.00
8	Victorgrain (old strain)	5353	[Norton 20-93 (Big Boy) × Navarro] × Victoria	99	4	16	1600	35	0.07
9	Victorgrain 48-93 (new strain)	5355		99	7	25	2500	22	0.00
10	Victoria	2401	*Avena Byzantina*	95	1	6	600	77	0.00
		Totals and averages		93.9	25	92	9200	37	0.10
			B. Known Resistant						
11	Bond		*Avena sterilis* × *A. Sativa* (var. Golden rain)	90	1	6	600	10	90
12	Bonda	4329	Bond × Anthony	97	2	5	500	2	98
13	Camellia	4079	Bond × Alber	96	2	7	700	5	95
14	Carolina Red	4313	Nortex × Victoria	95	1	4	400	6	94
15	Clinton	3971	Iowa D69 × Bond	99	2	7	700	1	99
16	Delair	4653	Fulghum × Bond	99	2	7	700	1	99
17	Delta Red 88	4220	Ferguson 922	85	6	18	1800	18	82
18	Forkedeer	3170	Reselection from winter Fulghum (C.I. 2499)	83	2	6	600	17	83
19	Nortex 107	4847	Nortex	81	4	18	1800	20	80
		Totals and averages		91.6	22	78	7800	8.8	91.1

* Cereal Investigation Accession numbers of the Division of Cereal Crops and Diseases, U. S. Department of Agriculture.
† The uninoculated seeds used as checks in these tests about equalled in number those of the inoculated.

every case the seeds from the known susceptible varieties obtained from commercial sources proved more than 99 percent susceptible. Of 9200 seeds of susceptible varieties tested, in 25 trials with a total of 92 replications, there were found only nine seeds, or 0.1 percent, that developed healthy roots following the treatment. Some of these seedlings were set in pots in the greenhouse and remaind healthy through maturity. The evidence points to these individuals being resistant and not escapes, and may be assumed for the present to represent mechanical mixtures within the commercial seed lots employed. On the other hand, none of the 7800 seeds of the nine resistant varieties tested, showed evidence of susceptibility (Table 1).

It was also noted that the susceptible varieties differed among themselves in regard to the incidence of non-germinating seeds following the treatment. Thus the varieties Mustang, Arlington, and Stanton showed much lower outright "kill" than Victoria, Letoria, Vicland, and some others. These differences seem to correlate with some field observations made on the relative susceptibility of the different Victoria cross oats.[1]

Carolina Red is included among the resistant oats in Table I. This variety shows high resistance to the blight under field conditions and also by the laboratory test. It represents another example of a Victoria-derived oat that is resistant to Victoria blight.[11, 12] In this connection it should be remarked that the type of crown rust resistance this variety exhibits is not characteristically of the Victoria type. The reaction of this oat to the prevalant crown rust races in Mississippi, including race 45, seems to be intermediate between that of its two progenitors, Nortex and Victoria, but somewhat nearer to Nortex.

Discussion

Some interpretations of the workings of the method may be helpful. During the early attempts, results indicated that that toxins produced by the fungus in liquid media, alone were capable of slowing down or inhibiting the germination of the seeds. Toxins produced by *H. victoriae*[9] have been reported to affect oat plants at very low dilutions[7]. The use of the toxins without the fungus did not produce the desired results (quick mass killing or inhibition of the germinating mass of seeds) because some of the temporarily inhibited seeds later developed as more or less normal seedlings. The fungus on the other hand produced much better growth on the seed when introduced with the nutritive agar which apparently sustained its growth. The nutritive agar may also have supplied the factor of continuity in the infective and toxic processes in regard to the seed, because it serves as the constant source, from the start and for some days afterwards, of both penetrating hyphae and toxins. The concentration of fungus and toxins in the agar seemed of the greatest importance because any further dilution of the agar with water, from the determined ratio 1:2, gave unsatisfactory results.

The apparent predisposing influence of the agar medium itself upon the seed may be supposed to be due to partial asphyxiation. This may also explain the slight stunting effect the agar medium produces, with or without the fungus, on young sprouts of some resistant varieties.

The temperature factor most likely aided the growth of the fungus, and perhaps the amount of toxins produced, and the rate of penetration of these toxins into the vital parts of the seed.

It is suggested that this method be also tried with other fungus pathogens, particularly with certain ones causing similar root and basal stem rots, such as *Pythium, Phytophthora, Penicillium* and other species.

Summary

A laboratory or "rag-doll" method of testing resistance of oats to Victoria blight (*Helminthosporium victoriae*) is described. This method differs from the prevalent field and greenhouse methods chiefly in that the test is conducted in the seed stage and is completed in the laboratory in about five to seven days, before or soon after the seeds have sprouted. The method is simple, and is particularly adapted to the testing of large lots of seed in bulk with speed, uniformity, and a high degree of accuracy. Essentially, it consists in coating the seeds with an agar culture of the casual organism mixed with water in the proportion of 1 to 2, keeping the treated seeds for five to seven days in "rag-dolls" at 100 percent relative humidity, and at a temperature of 25° to 30°C. Replicated tests with 17,000 seeds belonging to nine resistant and nine susceptible varieties showed the accuracy of the method to be very high. Plant breeders may employ this method for selecting disease resistant individuals with ease, confidence, great economy

of time, and with only negligible expense in ordinary equipment.

Literature Cited

1. ATKINS, I. M. *Texas Agr. Exp. Sta. Bul.* 728. 10pp. 1950.
2. COFFMAN, F. A., H. A. RODENHISER, and F. W. TAYLOR. *Agron. Jour.* 41:551-554. 1949.
3. DICKSON, J. G. Diseases of Field Crops. McGraw-Hill Book Co., New York, 1947.
4. IVANOFF, S. S. *Phytopath.* 40:13-14. 1950.
5. ————. *Bot. Gaz.* (In press).
6. LITZENBERGER, S. C. *Iowa Res. Bul.* 370. 453-496 pp. 1949.
7. ————. *Phytopath.* 39:300-318. 1949.
8. ————, and H. C. MURPHY. *Phytopath.* 37:790-800. 1947.
9. MEEHAN, FRANCES, and H. C. MURPHY. *Science* 104:413-414. 1946.
10. MURPHY, H. C., and FRANCES MEEHAN. *Phytopath.* 36:407. 1946.
11. POEHLMAN, J. M., and C. H. KINGSOLVER. *Agron. Jour.* 42:498-502. 1950.
12. ROSEN, H. R. *Phytopath.* 39:20. 1949.
13. STAMPER, E. R., JOHN G. ATKINS, and JOHN P. GRAY. *Louisiana Agr. Exp. Sta. Bull.* 431: 14pp. 1948.
14. STANTON, T. R. *Jour. Am. Soc. Agr.* 40:751-757. 1948.
15. ————, H. C. MURPHY, F. A. COFFMAN, and H. B. HUMPHREY. *Phytopath.* 24:165-167. 1934.

(*Received July 30, 1951*)

◆

THE GROWING BACKLOG OF GENETIC DEFECT

At the second annual meeting of the American Society of Human Genetics, Dr. H. J. Muller explored in detail the widely current view that the build-up of the pool of human genes for sundry defects and deficiencies is so slow that it may be ignored. He concludes that the changing survival patterns of modern man drastically upsets the selective force of differential mortality which in the past kept many deleterious genes at a low frequency. The extent to which changing survival patterns have abrogated any selection prior to reproduction is not generally realized. In the United States of ten female babies born, eight survive through the reproductive decades; in India two out of ten survive.

The following brief quotation gives only a very inadequate sample of a statement which deserves a much wider audience than it has yet achieved.—EDITOR.

WE see that, in mankind under present circumstances, these heterozygous genetic weaknesses, when in a setting of favorable external conditions, are seldom to be classed as outright "diseases." In view of this, we might in a very limited sense agree with the statement quoted in our introduction, that "mutation as a direct cause of disease is extremely rare and of little practical significance." This situation is however due to the fact, so fortunate for all of us in this generation, that our germ plasm was selected, in our more primitively-living ancestors, for a world without central heating or refrigerators, without labor-saving mechanisms in the home, in industry or in agriculture, without sewers or bathrooms, and without knowledge of contraceptives, asepsis, antibiotics, calories, vitamins, hormones, surgery or psychosomatic treatment. And so now for the first time, with the newly found aid of all these devices and methods, the average American, in spite of his eight or more inborn disabilities, adding up to at least a 20 percent natural disadvantage, manages to get by for almost the three score and ten years which, surprisingly enough, ancient tradition declared to be the "normal" span.

In view of the considerations adduced in the preceding section, the question now arises: granting this inborn disadvantage which would amount to at least 20 percent under primitive conditions, may we not regard this as relatively unimportant under our present conditions of living? Furthermore, may we not confidently expect that, with continued advances in general technology, living standards and medicine, the genetic burden will be further lightened and kept very small indeed?

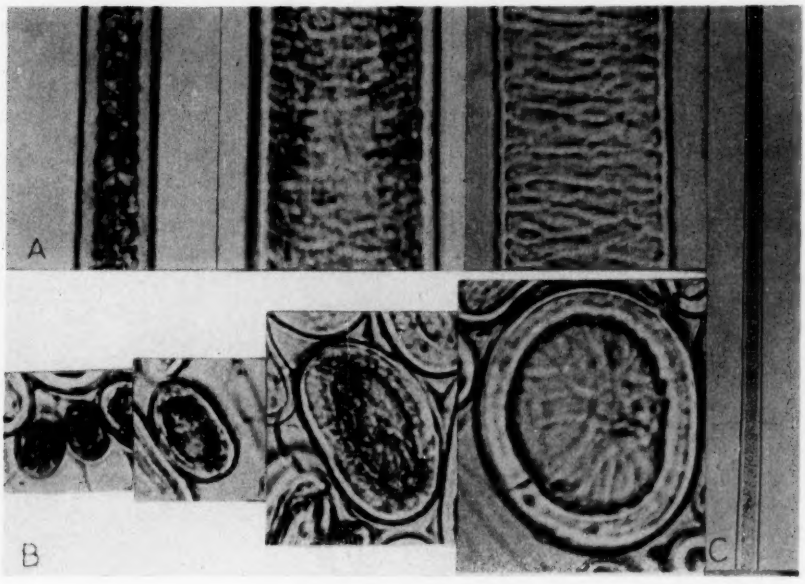
* * *

The great trouble with this method is that if (as today) it is unaccompanied by artificial selection it passes down to an indefinite number of future generations the burden that it has spared the treated generation itself. Of course these later generations can be treated in turn. But each successive generation will have not only the mutant genes which have in this way been passed along to it but also its own new crop of mutations. Thus the number of mutant genes will increase unless and until we again let as many die out as arise. To put the matter in other words, if our ameliorative procedures succeed they must inevitably (barring conscious selection) cause a smaller number of mutant genes to be reproductively eliminated per generation than were eliminated originally. In fact that is today one of the main

(*Continued on page* 240)

WHITISH, A MODIFIER OF CHOCOLATE AND BLACK HAIRS IN GUINEA PIGS

HEMAN L. IBSEN AND BETTY LANNING GOERTZEN*



DISTRIBUTION OF PIGMENT

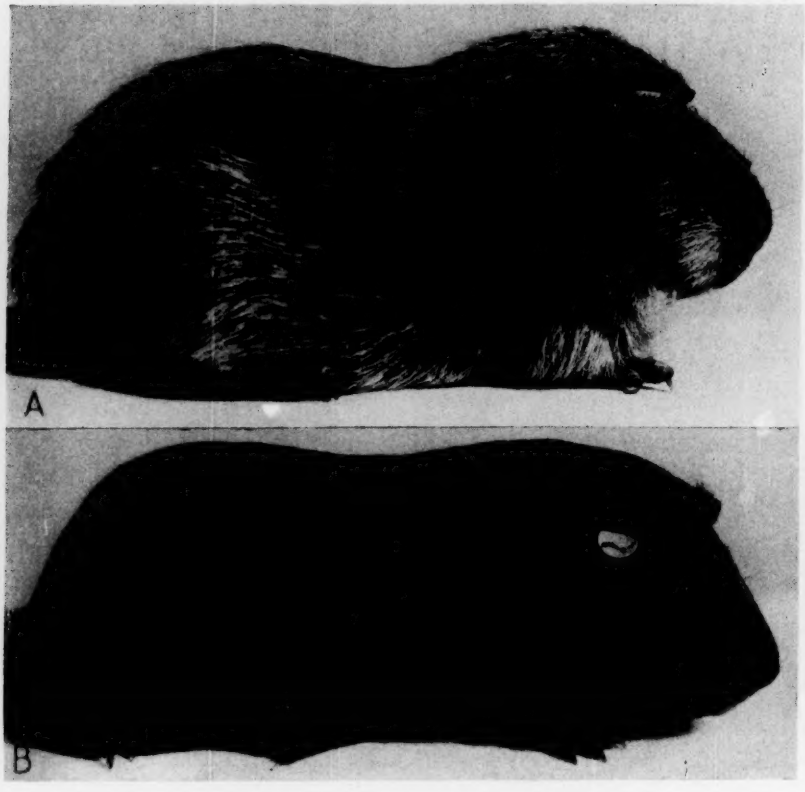
Figure 7

Microphotographs of side views of whole hairs (*A*) and of cross sections of hairs (*B*) of very whitish chocolate guinea pigs (*IWbb*). On the left are shown preparations from near the tips of the hairs. Large numbers of intensely pigmented chocolate granules are present. A few millimeters below the tip, (the second section shown in *B*) reveals only a medium distribution of chocolate pigment granules, with the granules present in the cortex concentrated close to the medulla. A few millimeters farther toward the base (third section), the chocolate granules are much fewer in number, and are still concentrated near the cortex-medulla interface. The base of the hair (right) shows no pigment granules either in side view or in cross section. *C* shows a side view of such a hair.

A GENE, "white tipping," affecting hair color was described by Ibsen.[2] It was assumed the gene was incompletely dominant to its allele for normal hair color but no data were furnished to support the assumption. Later, Wright, [3, 4] reported a similar character which he called "dinginess."

Wright called attention to the fact that white tipping is not a satisfactory name for the character he described since very few if any of the hairs actually have white tips. On the other hand, dinginess does not adequately describe the character as found in our animals (Figures 8 and 9). The latter have a silver appearance due to the large number of hairs that have subterminal white, or nearly white, bands. For that reason we call it "whitish."

* Contrbution No. 167, Department of Animal Husbandry, Kansas State Agricultural Experiment Station, Manhattan, Kansas.



VARIATIONS IN "WHITISH" GUINEA PIGS

Figure 8

Two grades of whitishness are recognized in this study. The extensive white coat in the chocolate guinea pig (*A*) is homozygous for the *W* gene (*WWbb*). Chocolates displaying less white are heterozygous (*Wwbb*).

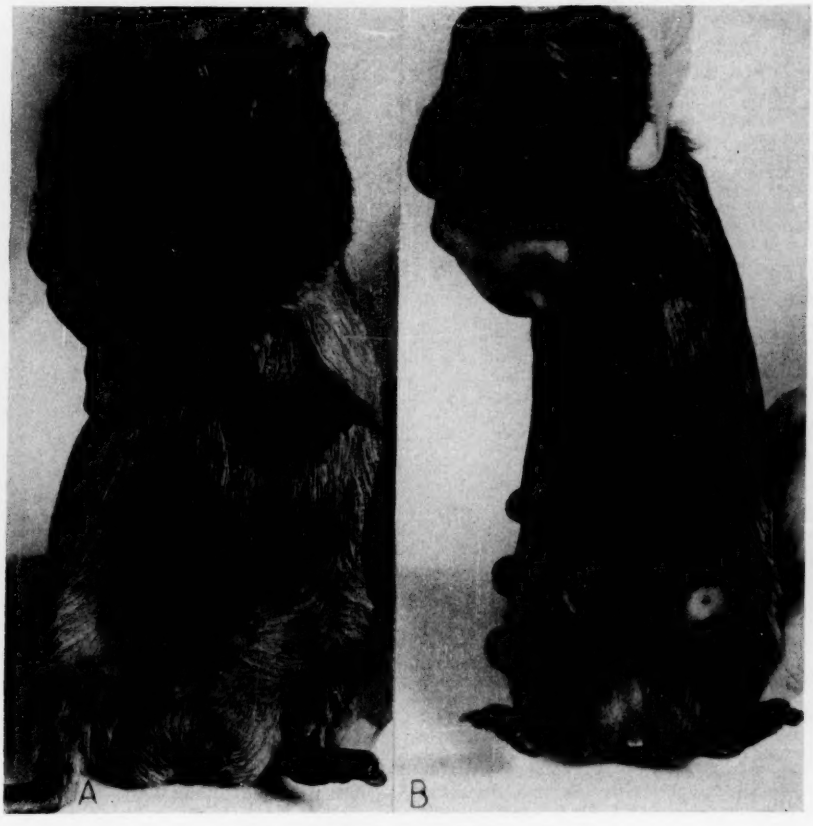
Method of Studying Whitish Hairs

Instead of using a grading system like Wright, we have made it a practice to store in paper envelopes representative hair samples from most of the animals. Several samples have been taken at different times from each animal to determine the possible effects of age and sex on the expression of the character. The samples are the basis for the microscopic study, as well as the macroscopic.

The Hardy device[1] was used in making cross sections for the study of variation in the distribution of pigment granules in whitish hairs. A microscopic examination was made of several hundred hair samples. These hair samples were taken from the head and back of each animal. A miniature diagrammatic outline of the animal on each envelope was used to indicate the locations from which the samples were taken. Each envelope also had written on it the pedigree number of the animal, the date at which the sample was taken, and a description of the animal. The samples were placed carefully in the envelope so that they would have practically the same appearance as they did on the living animal.

Description of Whitish Chocolates

The pigment at the tip of the hair is of full intensity, but the depth varies considerably. Some tips are only one millimeter wide



WHITE EXTENDS OVER ENTIRE COAT

Figure 9

Ventral view of two animals shown in Figure 8.

while others may be as much as 15 mm. in width (Figures 10*B* and 10*F*). Some of the hairs are completely white below the tip, a condition not found in Wright's stock. Other hairs, different from any described by Wright, have a completely white band under the pigmented tip, and this is followed by a dilute brown band to the base of the hair. In other hairs there are three bands below the tip, first a completely white one, followed by a dilute brown, and this in turn by another white band at the base of the hair (Figure 10*C*).

In the extremely whitish animals most of the hairs are white along the entire length below the chocolate pigmented tip (Figure 10*B*). As would be expected, the less whitish animals have fewer of this kind of hair, and those few hairs that are white at the base usually have a wide pigmented tip (Figure 10*F*).

The classification of the animals at this laboratory was based only on the presence or absence of subterminal white. None of Wright's dingy animals had hairs with subterminal white, but many of our whitish animals showed the dingy character. For example, some of our whitish animals have hairs which are an intense chocolate at the tip, and this is followed by a pale brown extending completely to the base (Figure 10*A*). Other hairs, in the same animal, have the dark chocolate tip followed by two dilute brown bands with a darker brown between, the dilute band near the tip being less dilute than the others

(Figure 10*D* and *G*). Still other hairs have three dilute brown bands with darker brown bands between (Figure 10*E*). Although there were more subterminal bands occurring in our stock than were described by Wright, these animals probably have the dingy character.

Description of Whitish Blacks

Wright never found blacks that showed the dingy character. Our blacks were mated to very whitish chocolates and the black progeny backcrossed to the whitish chocolates in an attempt to produce whitish blacks. Blacks, with a few whitish hairs on the head, were produced. A few of these hairs were found to be white except for the black pigment at the tip, but most of the affected hairs are banded, having either a dilute black or a white band proximal to the intense black tip with a less dilute black extending below the band to the base. Whitish disappeared with age in blacks in every case examined. In most cases whitish was still present at two months, when the first hair sample was taken, but disappeared before the end of the first year. In all blacks that showed whitish the color of the animal was not an intense black. The animal had the appearance of an intense chocolate as long as whitish was present. With the disappearance of whitish with age the black became more intense.

Inheritance of Whitish

In order to study the inheritance of whitish, matings were made involving animals that either did not show the character, showed it in extreme expression, or showed it to a much less degree. The progeny obtained from these matings indicate that whitish is due to an incompletely dominant gene (*W*), with modifying factors affecting its appearance.

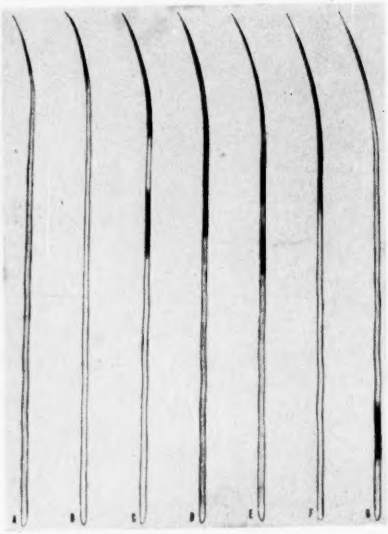
Like Wright's dingy character the animal must carry *C*, the gene for full color, in order for whitish to show. The genes c^a, c^r, and c^d when expressed were epistatic to whitish.

Red hair is unaffected by *W*. When whitish occurred in chocolate-and-red animals the red never contained whitish hairs.

In one case observed the whitish did not become visible until after the animal was two months old. Neither parent showed such a delay in expression of whitish. All other animals were whitish at birth.

Whitish was expressed only in the blacks homozygous for whitish (*WW*), and not always then. When *WW Bb* blacks and *WW bb* chocolates were mated all but three of the chocolate progeny showed extreme expression of whitish, but at least a third of the blacks did not show whitish (Table I). The three chocolates showing decreased whitish apparently carried a minus modifier which was present in the stock and affected all of the matings. It is quite evident that there are modifiers affecting this character since both the *WW* and *Ww* animals show a great deal of variation in the amount of whitish expressed. Of 70 blacks produced from this mating, 42 showed whitish and 28 did not. These 28 blacks would be expected to be *WW*. Unfortunately, none of these animals was mated.

Selection was carried on for a number of generations in the production of blacks that



BANDING PATTERNS

Figure 10

Diagrammatic drawing of typical hairs found in whitish chocolate guinea pigs (*W-bb*). The different shades of black in the drawing represent the different shades of brown (chocolate) pigment found in the hairs. All of the hairs have dark brown tips varying considerably in the depth of the brown pigment. *A*—All of the hair below the dark brown tip is a uniform dilute brown. *B*—All of the hair below the tip is completely white. *C*—Below the dark brown tip there are two white bands with a medium intense brown band between. *D*—Below the dark brown tip there are two dilute brown bands with a medium intense brown band between. *E*—Has five alternating light and dark brown bands below the dark brown terminal band. *F*—Corresponds to *B* except that the dark brown pigment in the tip extends to a greater depth, making the hair less whitish in appearance. *G*—is similar to *D* except that the medium intense brown band is relatively narrow and nearer the base of the hair. Figure 10*A*, and possibly 10*D*, *E*, and *G* are similar to the types of hair reported by Wright as being present in his "dingy" animals.

showed whitish. That selection had an effect is evident from the results. In the first 35 blacks produced, 17 expressed whitish and 18 did not. Of the last 35 blacks produced, 25 showed the character while 10 did not. The numbers are quite small, but they do indicate an increase in the number expressing the character when selection was practiced. Only two matings were made between whitish blacks (*WW*), but all four black offspring showed the character.

There is a sex-limited effect in chocolates in that most females gradually lose whitish while males tend to retain it.[2] From the 124 hair samples observed, 22 females showed persistence of whitish while 41 did not, and 42 males showed persistence while 19 did not. A high chi-square value indicates a sex difference in the persistence of whitish (Table II). Some matings were made in which both chocolate parents and offspring could be checked for persistence (Table III). The numbers are small, but they do indicate the possibility that persistence is due to a partially sex-limited dominant gene. The female, in this case, would have to be homozygous for the gene in order to retain whitish with age, while males could be either homozygous or heterozygous for the gene.

In non-persistence, whitish begins to disappear before the animal is six months old, and it usually is lost completely before the end of the first year. Even after complete disappearance of whitish the chocolate hairs never attain an intense color. In persistent animals the whitish usually is decreased slightly with age but never is lost completely.

In Table IV the six monohybrid matings involving chocolates are tabulated. All of the progeny from homozygous dominant parents (*WW*) showed whitish (Table IV, row 1). Eight of the offspring did not show as extreme whitish as the others. These eight were classified as heterozygotes, but it is more probable that they are homozygous dominant for whitish with a modifier or modifiers reducing the expression of the character. The remaining 229 progeny showed extreme expression of whitish (*WW*).

When homozygous dominant (*WW*) were mated to heterozygotes (*Ww*) the observed ratio was 36 extreme whitish (*WW*) to 54 with less whitish (*Ww*) (Table IV, row 2). The chi-square value is not significant; however it is near the five percent level. All of the animals did show whitish however, and here again, it is possible that some of the homozygous dominants were classified as heterozygotes due to the action of a minus modifying gene.

A few matings were made between animals that showed extreme expression of whitish (*WW*) and those which did not show whitish (*ww*) (Table IV, row 3). Eleven progeny were heterozygous in appearance (*Ww*) with one showing only dinginess (*ww*). This one dingy had been classified as heterozygous for whitish (*Ww*). It is possible that due to the non-persistent gene, whitish disappeared before the hair sample was taken. It is also possible that this hair sample did not include whitish hairs, although they may have been present on the animal.

When both animals mated showed a less extreme expression of whitish (*Ww*) the observed ratio was 16:42:20 (Table IV, row 4). This ratio fits the theoretical ratio, 19.5:39:19.5.

When heterozygotes (*Ww*) were mated to recessive animals (*ww*), 20 recessives (*ww*) and 30 heterozygotes (*Ww*) were obtained (Table IV, row 5).

Table I. *Progeny from matings of* bb *blacks with a few whitish hairs* (WW) *and extremely whitish chocolates* (WW).

ROW NO.	COLOR OF PROGENY	SEX	NUMBER	TOTAL
1	VERY WHITISH CHOCOLATES (WW)	MALE	42	91*
		FEMALE	49	
2	WHITISH BLACKS (Ww)	MALE	19	42
		FEMALE	23	
3	BLACKS NOT SHOWING WHITISH (ww)	MALE	19	28
		FEMALE	9	

* Three were not extremely whitish.

Table II Data showing a sex difference in persistence of whitish in chocolates expressing the character (W).

ROW NO.	CHOCOLATES		MALE	FEMALE	TOTAL
1	PERSISTENT	observed number	42	22	64
		expected number	32	32	
2	NONPERSISTENT	observed number	19	41	60
		expected number	30	30	
	TOTAL		61	63	124

Table III Data showing the persistent and nonpersistent offspring produced when persistent males were mated to nonpersistent females, persistent males to persistent females, and nonpersistent males to nonpersistent females.

ROW NO.	PARENTS	OFFSPRING			
		PERSISTENT		NONPERSISTENT	
		MALE	FEMALE	MALE	FEMALE
1	Persistent ♂ x Nonpersistent ♀	12	3	4	11
2	Persistent ♂ x Persistent ♀	6	7	0	5
3	Nonpersistent ♂ x Nonpersistent ♀	1	0	1	1

Table IV A tabulation of the six monohybrid whitish matings showing the observed and expected ratio of the extremely whitish (WW), slightly whitish (Ww), and non-whitish (ww) progeny produced. All parents and offspring are chocolates (bb).

ROW NO.	PARENTS	OFFSPRING			
			WW	Ww	ww
1	WW x WW	observed number	229	8	
		expected number	237		
2	WW x Ww	observed number	36	54	
		expected number	45	45	
3	WW x ww	observed number		11	1
		expected number		12	
4	Ww x Ww	observed number	16	42	20
		expected number	19.5	39	19.5
5	Ww x ww	observed number		30	20
		expected number		25	25
6	ww x ww	observed number		4	102
		expected number			106

Four offspring were obtained that showed a slight expression of whitish (*Ww*) when both parents were assumed to be recessives (*ww*) (Table IV, row 6). These four offspring resulted from three matings. A possible explanation is that one of the parents in each mating carried $c^r c^r$, or some other factor, which was not observed but which inhibited the expression of whitish. Since the inhibitor was not observed, these animals would be classified as recessives. The remaining 102 offspring from the mating did not show whitish (*ww*).

Chocolate (*bb*) animals expressing c^r, c^d, or c^a are not included in Table IV since whitish is hypostatic in these animals and their genetic composition cannot be determined by their appearance.

Discussion

None of Wright's dingy animals had hairs with subterminal white (*W*). This is probably because he was working with inbred stock which had lost the gene, and were therefore of the composition *ww*. Many of our animals, on the other hand, showed the dingy character. This was especially true of those that were heterozygous for *W*. The *WW* animals had so many whitish hairs that only a small number could show the dingy character. Even here there were a few whose non-whitish hairs were an intense chocolate color along their entire length. All dingy animals that showed no subterminal white hairs were classified as *ww*. But among the *ww* animals were also some that did not show any dinginess.

Although, as shown above, whitish and dingy are frequently found in the same animal there is no evidence at hand to determine whether or not they are linked. Another possibility is that dinginess and whitish belong to the same "complex" since they are both hypostatic to the same genes (c^d, c^r, c^a), with whitish being the most striking in its effect.

Summary

Whitish, a gene influencing hair color, occurs with some variation in chocolates and blacks, but chiefly in chocolates. An incompletely dominant gene (*W*) causes the whitening or extreme paling of the hair several millimeters below its tip. The animal must carry *C*, the gene for full color, in order for whitish to be expressed. Red hair (*CCee*) is entirely unaffected by *W*. In some animals modifying factors are present which reduce the expression in homozygotes (*WW*) and heterozygotes (*Ww*) making it difficult to classify the animals correctly. For this reason animals classified as heterozygotes (*Ww*) by appearance may actually be homozygotes (*WW*).

The effect of *W* on black hair (*B*) is very slight; some of the homozygotes (*WW*) have a few whitish hairs on the head, while heterozygotes do not show any whitish. All blacks lose whitish with age. A gene for persistence of whitish may be present in blacks, but it is never expressed. In chocolates most of the females lose whitish while the males tend to retain it. The persistence of whitish seems to be due to a partially sex-limited dominant gene.

Literature Cited

1. Hardy, J. I. *U. S. Dept. Agri.* Circular 378. 1935.
2. Ibsen, H. L. *Proc. 6th Int. Congress. Genetics* 2:97-101. 1932.
3. Wright, S. *Biol. Symp.* 6:337-355. 1942
4. ————. *Genetics* 32:115-141. 1947.

(*Received August 1, 1949*)

Bacon on the Light of Reason

THE human understanding is no dry light, but receives an infusion from the will and affection; whence proceed sciences which may be called "sciences as one world," for what a man had rather were true, that he more readily believes. Therefore he rejects difficult things from impatience of research; sober things because they narrow hope; the deeper things of nature from superstition.—Francis Bacon. *Novum Organum. 1620.*

MULLER'S METHOD OF CALCULATING POPULATION SIZES

For Synthesizing New Stocks or Lines

G. B. MAINLAND*

IN many breeding programs, both applied and theoretical, the behaviors of the genetic factors are known prior to their combinations into new stocks or lines. From the standpoint of efficiency of labor, time, and space, it is desirable to determine the number of matings to be made, the number of progenies to be raised, or the number of progenies to be tested in order to have a 95 or 99 percent probability of obtaining or identifying one or more individuals of the desired genetic combinations. On a number of occasions, the writer has noted the raising, testcrossing, or selfing of excessive numbers of individuals when a smaller number would have been sufficient to have given one or more individuals of the desired genetic type at a 95 or 99 percent probability level.

In 1923 Muller[2] presented an approximate formula for calculating the number of individuals to be raised or tested for a desired probability of success. This very useful formula has been almost completely overlooked; and, in the writer's opinion, the formula deserves reemphasis.

J. F. Crow (private communication) and the writer independently and each by different methods developed the same formula in a slightly different form from that presented by Muller only to discover that he had published the formula more than 25 years previously.

Muller's modified formula† is as follows:

$$n = k\ (r + \tfrac{1}{2})$$

where, n = the number of individuals to be raised or tested,

$k = -\log_e (1 - p)$ with p equalling the desired probability level,

and $r = a/b$, the ratio between the theoretically expected proportion of non-desired types, a, and the theoretically expected proportion of desired types, b.

Since the evaluation of k for the non-mathematical biologist may prove somewhat difficult, a short table of probability levels and the corresponding k values to the nearest one-hundredth is presented here (Table I). However, for practical purposes, the first decimal is sufficient inasmuch as it is necessary to round off the n values to a whole number of individuals. Many workers also prefer to use the whole integer immediately larger than the calculated value of n, thereby slightly increasing the probability of obtaining or identifying the desired genotype.

Since the 95 and 99 percent probability levels are the commonly accepted criteria for biometrical work, only the corresponding k values of 3.0 and 4.6, respectively, will be generally employed.

An example of the use of the formula in a breeding program is presented since the uses of the calculated population sizes for various genetic ratios and probability levels may not be familiar to all readers.

> ***Problem:*** Starting with one line that is *AA bb* and with another line that is *aa BB*, one desires to synthesize and identify the homozygous strain *AA BB* with a 99 percent probability of success at each step in the breeding program. *A* and *B* are known to assort independently.

Among the F_2's to be obtained from the cross between the two lines, theoretically 1/16th will be *AA BB*, that desired, whereas 15/16ths would be of non-desired genotypes. Therefore,

$$r = 15.$$

And since a 99 percent probability is desired,

$$k = 4.6.$$

Substituting these values in the modified Muller equation,

$$n = k\ (r + \tfrac{1}{2}), \text{ then}$$
$$n = 4.6 \times (15 + \tfrac{1}{2}), \text{ or}$$
$$n = 4.6 \times 15.5, \text{ and}$$
$$n = 71.3.$$

*Contribution from the Department of Zoology-Entomology, Alabama Polytechnic Institute, Auburn, Alabama. The author acknowledges the helpful suggestions of J. F. Crow, C. F. Poole, and A. L. Tester in regard to the preparation of this manuscript.

†Muller's formula is $n = -log_e F\ (p - \tfrac{1}{2})$ with $-log_e F$ equalling k, and p always being 1 greater than r.

Hence, one determines that 72 F_2 individuals should be raised in order to have a 99 percent probability that there will be one or more individuals of the genotype *AA BB* among the F_2 progeny.

Among the F_2 individuals phenotypically *A— B—*, 1/9th are expected to be *AA BB* whereas 8/9ths are expected to be of other genotypes. Similarly evaluating n for $r = 8$, one determines that 39 (39.1) individuals of the double dominant phenotype should be tested by either inbreeding or testcrossing so that there will be a 99 percent probability of including one or more individuals of the desired genotype, *AA BB*, in the test.

Among the F_2 individuals phenotypically *A— B—*, the following obtains with respect to the phenotypic segregation of their F_3 and testcross progenies:

F_2 Genotype	F_3 Ratios	Testcross Ratios
AA BB	all *AB*	all *AB*
AA Bb	3*AB*:1*Ab*	1*AB*:1*Ab*
Aa BB	3*AB*:1*aB*	1*AB*:1*aB*
Aa Bb	9*AB*:3*Ab*:3*aB*:1*ab*	1*AB*:1*Ab*:1*aB*:1*ab*

Although the last genotypic group gives an F_3 segregation ratio of 9:3:3:1 or a testcross ratio of 1:1:1:1, it will give an F_3 segregation of 3:1 or a testcross segregation of 1:1 with respect to either pair of alleles. Therefore, $r = 3$ for an F_3 test, and $r = 1$ for a testcross; hence the evaluations of n for these ratios are respectively 16 (16.10) and 7 (6.90). Consequently, it is necessary to raise either 16 F_3 or 7 testcross individuals from *each* of the 39 F_2 double dominants in order to have a 99 percent probability of detecting heterozygosity for either *A* or *B* in each individual tested. If no segregation occurs among the progeny of a given individual under these test conditions, there exists a 99 percent probability of that individual being the desired type, *AA BB*.

Mathematical Development

Since neither the derivation of Muller's approximate formula nor that of the exact logarithmic formula derived by Mather[1] from the binomial theorem is presented in the genetic or breeding literature, it appears desirable to derive both equations therefrom and to relate them.

Let a = the theoretical proportion of the non-desired types (phenotypes or genotypes), and
b = the theoretical proportion of the desired type:

therefore, $a + b = 1$. (2)

And let $r = a/b$, (3)

n = the number of individuals to be raised or tested,
p = the probability of occurrence of one or more individuals of the desired genotype among a group of n size, and
q = the probability of occurrence of only individuals of the non-desired genotypes among a group of n size;

therefore, $p + q = 1$. (4)

Hence, $(a+b)^n - a^n = p$, or (5)

$1 - a^n = p$, or (6)

$a^n = 1 - p$.* (7)

To evaluate for n, it is necessary to transform Equation 7 into logarithmic form, viz.

$$n \log a = \log (1 - p), \text{ or} \quad (8)$$

$$n = \frac{\log (1 - p)}{\log a}, \text{ or} \quad (9)$$

$$n = \frac{\log q}{\log a} \quad (10)$$

If it is desirable to evaluate n from p, the probability of obtaining one or more individuals of the desired genotype, and from b, the expected proportion of the desired genotype, Equation 10 becomes

$$n = \frac{\log (1 - p)}{\log (1 - b)} \quad (11)$$

(Both Equations 10 and 11 are exact methods for evaluating n at any desired probability level.)

Since $a = 1 - b$, or (See 2)

$$a = \frac{r}{r + 1} \quad (12)$$

because $a = \dfrac{a\ (1/b)}{1/b}$ (13)

$$a = \frac{a/b}{1/b}, \quad (14)$$

but $a = \dfrac{r}{\left(\dfrac{a + b}{b}\right)}$, (from Equation 3) (15)

$$a = \frac{r}{\dfrac{a}{b} + 1}, \text{ (from Equation 3)} \quad (16)$$

$$a = \frac{r}{r + 1} \quad \text{Q.E.D.}$$

By substituting $\dfrac{r}{r + 1}$ for a or $1 - b$ in Equation 10 (or 11), the formula then becomes

$$n = \frac{\log (1 - p)}{\log \left(\dfrac{r}{r+1}\right)} \quad (17)$$

*The author realizes that Equation 7 may be obtained directly; however, by relating the development to the binomial as in Equation 5, all relationships are obvious.

By converting to natural logarithms, Equation 17 becomes

$$n = \frac{\log_e (1 - p)}{\log_e \left(\frac{r}{r+1}\right)}, \text{ or} \qquad (18)$$

$$n = \frac{\log_e (1 - p)}{-\log_e \left(\frac{r+1}{r}\right)}, \text{ or} \qquad (19)$$

$$n = \frac{-\log_e (1 - p)}{\log_e (1 + 1/r)}. \qquad (20)$$

By letting $k = -\log_e (1 - p)$, Equation 20 then becomes

$$n = k\left[\frac{1}{\log_e (1 + 1/r)}\right] \qquad (21)$$

By substituting x for $1/r$, the denominator of Equation 21 is then expressed in the standard form of $log_e (1 + x)$, which is equivalent to the power series of

$$x - \frac{x^2}{2} + \frac{x^3}{3} - \frac{x^4}{4} + \frac{x^5}{5} - \frac{x^6}{6} \ldots\ldots$$

Hence, Equation 21 may be rewritten as $n =$

$$k\left[\frac{1}{\frac{1}{r} - \frac{1}{2r^2} + \frac{1}{3r^3} - \frac{1}{4r^4} + \frac{1}{5r^5} \ldots}\right] \qquad (22)$$

By dividing the power series into the numerator, *1*, Equation *22* becomes $n =$

$$k\left[r + \frac{1}{2} - \frac{1}{12r} + \frac{1}{24r^2} - \frac{19}{720r^3} \ldots\right] \qquad (23)$$

In Equation *23*, the power series is convergent and approaches the limit of $r + \frac{1}{2}$ with large values of r; furthermore, the third and subsequent terms alternate in sign. Consequently, the third and subsequent terms of the series may be neglected for practical purposes. Hence Equation *23* may be written in the following form:

$$n \doteq k\ (r + \tfrac{1}{2}) \qquad \text{Q.E.D.}$$

Discussion

Since Muller's formula (Equation 1) is approximate and the logarithmic formula (Equation 10) is exact, it is informative to note the error resulting from evaluating n by means of the approximate equation. Where $r = 1$, the error is *ca.* $+3.97$ percent; and where $r = 8$, the error of n is *ca.* $+0.11$ percent. With increasing values of r, the error decreases rapidly. In addition, the approximate arithmetic equation becomes somewhat more accurate than the logarithmic equation for high values of r if five-place logarithm tables are used for evaluating n by means of the latter equation.

In Table II are listed the values of n to the nearest one-hundredth at the two commonly used probability levels of 95 and 99 percent for some of the more common F_2, F_3 and testcross ratios. In the second and third columns, the n values are determined from the logarithmic equation, and the fourth and fifth columns from the modified Muller's arithmetic equation.

As noted previously, in all breeding work it is necessary to round off the n values to a whole integer. Therefore, it should be apparent that the error introduced by the use of the approximate equation is considerably less than that introduced by the intrinsic necessity of having to work with entire individuals in breeding programs.

The advantages of the modified Muller's arithmetic equation evaluated for k are twofold. First, once k is evaluated, this equation no longer requires the use of logarithmic tables for calculating n. Although the term *log q*, of the logarithmic equation may be similarly evaluated as a constant for the 95 and 99 percent probability levels, the logarithmic equation still requires the use of the logarithm of the variate, a, for the determination of n. Secondly, the variate, r, of the arithmetic equation normally is a whole integer, which can be determined easily, e.g. where the genetic ratio is 3:1, $r = 3$, and where the ratio is 15:1, $r = 15$, etc. On the other hand, the variate, a, of the logarithmic equation is either a fraction or its decimal equivalent. Consequently, it is apparent that the modified arithmetic equation of Muller evaluated for the desired probability level may be used with greater facility than the similarly evaluated logarithmic equation.

Summary

Attention is called to Muller's approximate equation for calculating with a specified probability of success: (1) the number of F_2 or

TABLE I. Probability Values and their Corresponding k Values.

Probability Value of Obtaining Desired Type	k Value
90%	2.30
95%	3.00
98%	3.91
99%	4.61
99.5%	5.30
99.9%	6.91

TABLE II. A Comparison of n Values Determined by the Logarithmic and Arithmetic Equations. (Six-place logarithms were used for the calculations of the n values in this table.)

Mendelian F_2, F_3, and Testcross Ratios	n Values (ex logarithmic) Probability Level 95%	99%	n Values (ex arithmetic) Probability Level 95%	99%
1:1	4.32	6.64	4.49	6.91
3:1	10.41	16.01	10.49	16.12
7:1	22.43	34.49	22.47	34.54
8:1	25.43	39.10	25.46	39.14
15:1	46.42	71.35	46.43	71.38
26:1	79.37	122.02	79.39	122.04
31:1	94.36	145.05	94.37	145.06
63:1	190.24	292.44	190.23	292.43
80:1	241.15	370.71	241.16	370.72
255:1	765.31	1176.47	765.41	1176.63

backcross individuals to be raised in order to obtain a desired type; (2) the number of individuals to be tested in order to include the desired genotype in the test; and (3) the number of individuals to be raised from an F_2 or testcross in order to identify the desired genotype. The use of the equation is illustrated. The approximate equation,

$$n = k\ (r + \tfrac{1}{2}),$$

is derived from the binomial theorem and is related to the exact logarithmic equation,

$$n = \frac{\log\ q}{\log\ a}$$

The exact method and Muller's approximate method are briefly contrasted in order to show that the two methods give very similar results.

Literature Cited

1. MATHER, K. The Measurement of Linkage in Heredity. Methuen & Co., Ltd., London. 1938.

2. MULLER, H. J. *Amer. Nat.* 57:66-73. 1923.

(*Received July 23, 1951*)

◆

THE GROWING BACKLOG OF GENETIC DEFECT

(*Continued from page 230*)

aims of these procedures. But the number eliminated originally (under primitive conditions) must have been the equilibrium number, i.e. equal to the number of new mutations arising. The number eliminated when ameliorative treatments are given is therefore less than the number of mutant genes arising. Thus the conditions for the application of the basic equilibrium theorem, $f = np$, have been violated. For we have diminished i, thereby increasing p, causing a rise in f. [i = the "selective disadvantage," = 1/p—ED.]

* * *

If the attempt were made to continue indefinitely to substitute a more remote equilibrium for f_1, by ameliorative practices, it would mean an ever greater heaping up of mutant genes. There would be no limit to this short of the complete loss of all of the genes or their degradation into utterly unrecognizable forms, differing chaotically from one individual of the population to another. Our descendant's natural biological organization would in fact have disintegrated and have been replaced by complete disorder. Their only connections with mankind would then be the historical one that we ourselves had after all been their ancestors and sponsors, and the fact that their once-human material was still used for the purpose of converting it, artifically, into some semblance of man. However, it would in the end be far easier and more sensible to manufacture a complete man *de novo*, out of appropriately chosen raw materials, than to try to refashion into human form those pitiful relics which remained. For all of them would differ inordinately from one another, and each would present a whole series of most intricate research problems, before the treatments suitable for its own unique set of vagaries could be decided upon.

Admitting this to be a *reductio ad absurdum*, our critics might object that no such unlimited continuance of mutational accumulation was intended, but only a reasonable amount of it, whatever that might prove to be. The answer to this is that unless the practice were indefinitely continued, there would be some stopping place (either sudden or gradual) and that, following this, elimination would after all have to be allowed to become equal in frequency to the new mutations, in order to prevent the still further accumulation of mutant genes.

* * *

It is very difficult to estimate the rate of the deteriorative genetic process which present practices occasion. No one knows how much less stringent selection is today, in any one particular, than it was in primitive times. But unless we take the naive position that ailments of genetic origin cannot be mitigated by artificial means we must admit that modern methods do result in the saving for reproduction of many mutant genes which otherwise would have been eliminated by the defects they produced. Thus, assuming only our 20 percent minimum value for the equilibrium frequency of genetic elimination, the fact that the average American now lives beyond 65 is a proof that nothing like the equilibrium quota is eliminated by death before the age of reproduction. This may also be deduced from the fact that an average number of children of not much more than two born per adult is at present about sufficient to maintain the population. [In the United States today, 2.2 children *per woman*—not per adult—is adequate to maintain population.—ED.]

—H. J. MULLER. Our Load of Mutations. *The American Journal of Human Genetics*, June 1950.

SYNDACTYLISM IN HOLSTEIN-FRIESIAN CATTLE

Its Inheritance, Description and Occurrence

F. E. Eldridge, W. H. Smith and W. M. McLeod*

A GRADE Holstein-Friesian heifer calf, born with only one toe on each of its front feet, was reported to the authors in November, 1948. Similar cases were found to have occurred in related animals from different herds in the same community, indicating that the characteristic might be hereditary. Some other related herds were then investigated and a sufficient number of cases was located to warrant an hypothesis concerning the mode of inheritance of this anomaly.

Bateson[1] in 1894 reviewed reports of several cases of syndactylism in cattle as well as in other members of the suborder *Artiodactyla*. The earliest of these reports reviewed by Bateson[1] was the work of H. Landois in 1881. In 1948 Kale[5] published a picture of a case of syndactylism in an Indian Zebu bull. These widely separated reports indicate that syndactylism in cattle is neither a new character nor one limited to only one species. In none of these cases, however, has there been presented data which would allow any hypothesis to be developed concerning the heredity of syndactylism in cattle.

Syndactylism is not uncommon in pigs, and according to Detlefsen and Carmichael[3] is inherited in that species as a simple dominant. They state that ". . . fusion may vary from complete, with no trace of separation on the hoof, to a less perfect fusion with two deep parallel lines of demarcation." Fusion was noted to be complete more often in the front feet than in the hind feet.

It has been demonstrated by Ross *et al.*[6] that syndactylism in pigs can result from malnutrition, also. The fact that this characteristic has been found to be induced either genetically or nutritionally emphasizes the need for caution in drawing conclusions concerning the basic cause for any departure from normal. In addition, these facts indicate a possible method for investigating the embryogeny of syndactylism in pigs, since either the presence of a specific gene or the occurrence of a certain state of malnutrition can cause the same anatomical malformation.

A great many cases of syndactylism have been reported in humans,[4] but the anatomical differences existing between a normal human hand or foot and a normal calf foot are so great that any similarities between the abnormalities are difficult to determine. It is of interest, however, to note that in the heritable cases of syndactylism in humans there has been considerable variation in expression.

Description

The first syndactylous heifer calf to be observed in this investigation appeared to have no impairment of movement at the age of five weeks as compared with normal footed animals, except that the single-toed feet provided less stable footing on a smooth, hard surface. Each of the single-toed feet lacked flexibility and had no more bearing surface than is found in one toe of a normal foot. By the time the heifer was about five months old, the left front hoof had curved both upward and outward at the tip, interfering to some extent with ease of movement. In general appearance the abnormal front feet were very similar to those in the photograph of the Zebu bull published by Kale.[5] The knees appeared to be larger than usual, but this may have been an illusion caused by the tapering of the front legs into the single toes. From all external appearances,

*Dairy Husbandman, Animal Husbandman, and Veterinarian, respectively, at the Kansas State College. This paper is Contribution No. *198*, Department of Dairy Husbandry; No. *174*, Department of Animal Husbandry; and No. *117*, Department of Veterinary Medicine, Kansas State College, Manhattan, Kansas.

FOUR LIMBS—SIX TOES

Figure 11

The syndactylous forefeet and didactylous hindfeet of the bull calf dissected in this investigation. Anatomical details of the structure are shown in Figures 13 to 15.

the rear legs and feet were perfectly normal. This animal, now 22 months old, is being kept by its owner as a milk cow, since she is from high producing stock and appears in all other ways to be normal and vigorous.

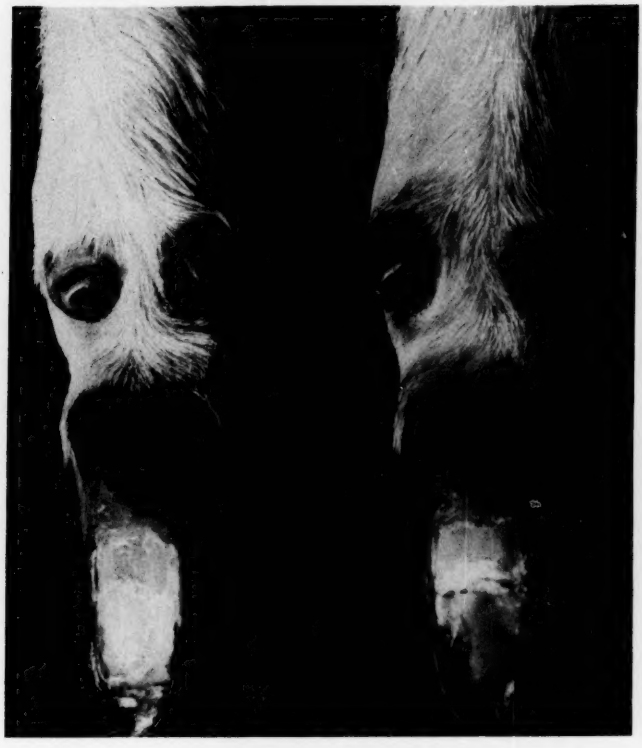
The second animal observed was a purebred Holstein-Friesian bull calf, on a different farm, but having some ancestors in common with the first one. (Figures 11, 12, 13, 14 and 15.) The bull calf had been observed alive and active by one of the authors, but died at about three months of age, the night before a trip was made to obtain detailed information. The cause of death was not determined, but there was little reason to suspect that the death was associated with the single-toed condition. It was mentioned by the owner, however, that the calf had never been so thrifty as the others, and, considering its age, was not so large as it should have been at the time of death. It had a coefficient of inbreeding of 0.21. The legs were removed from this bull calf and were brought to the College for detailed study.

Up to the time this paper was written the calf referred to was the only specimen which had become available for dissection.

The authors have observed considerable variation between calves in the expression of this character, but in outward appearance the syndactylous forelimbs of the above-mentioned calf were typical of a majority of the cases.

Before dissection, photographs were made of an anterior view of all four limbs, (Figure 11), and the bearing surfaces of the two front feet, (Figure 12). In addition, an X-ray photograph was made of the right limbs, (Figure 14). Immediately after dissection, photographs were made of the various bones of the right forelimb, (Figure 15), and after maceration the bones of both right limbs below the hock and knee were mounted and again photographed, (Figure 13). A detailed description has been made of the dissected right forelimb of this 3-month old calf in order to provide a basis for comparison of other calves found to be syndactylous. The right hindlimb, dissected at the same time, was found normal in all respects. The left foreleg and left hindleg were embalmed intact for demonstration purposes.

The hoof on each front foot of the calf, Figures 11 and 12, consisted of a wall and sole, similar to the hoof on any one of the main digits of a normal calf. Viewed from the front, the wall appeared almost symmetrical, with only a slight flattening on the medial side and with no evidence of a line of fusion. Posteriorily, the wall of the hoof was not reflected to



UNCLOVEN HOOFS

Figure 12

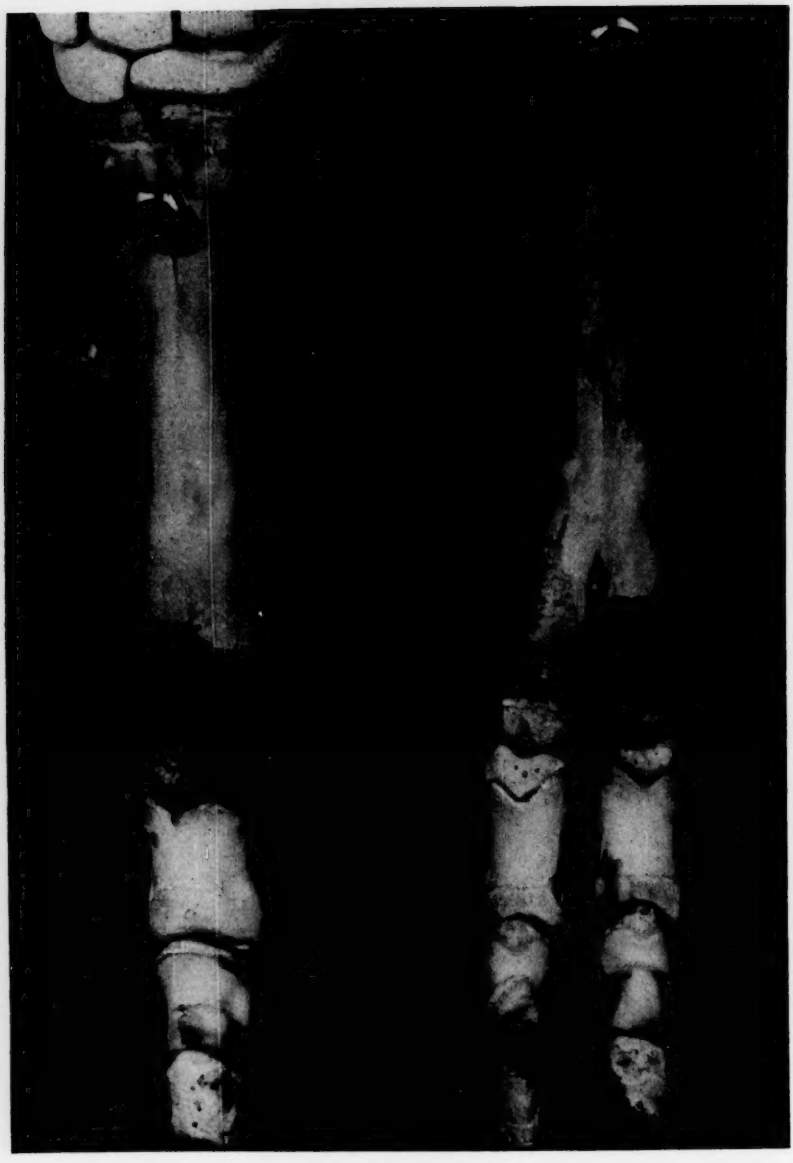
Soles of the feet of the syndactylous bull calf shown in Figure 11. Seventeen cases of syndactylism were reported in a Holstein population line-bred to certain ancestors identified as carriers of this defect.

form an angle and bars, as in the horse's hoof, but was replaced with periople which was continuous with the sole. The hoof was about equal in size to a normal foot when viewed from the side, i.e., from front to rear, but when seen from the front it was scarcely half as wide as a normal foot.

The dew claws were normal in location and appearance, the lateral one being larger than the medial one.

The *common digital extensor* was not divided into two branches at the fetlock joint and was inserted into the proximal end of the first phalanx, in contrast to the normal condition in which each of the two branches is inserted into the extensor process of its corresponding third phalanx. The *medial digital extensor* did not branch and appeared to be attached to the extensor process of the third phalanx in the medial plane of the limb. Sisso and Grossman[7] state that normally ". . . the *medial digital extensor* is inserted by two branches into the second and third phalanges of the medial digit." The *lateral digital extensor* was inserted into the lateral aspects of the second and third phalanges. This was as close to normal as could be approximated with only one digit. There were no reinforcing slips received from the suspensory ligament.

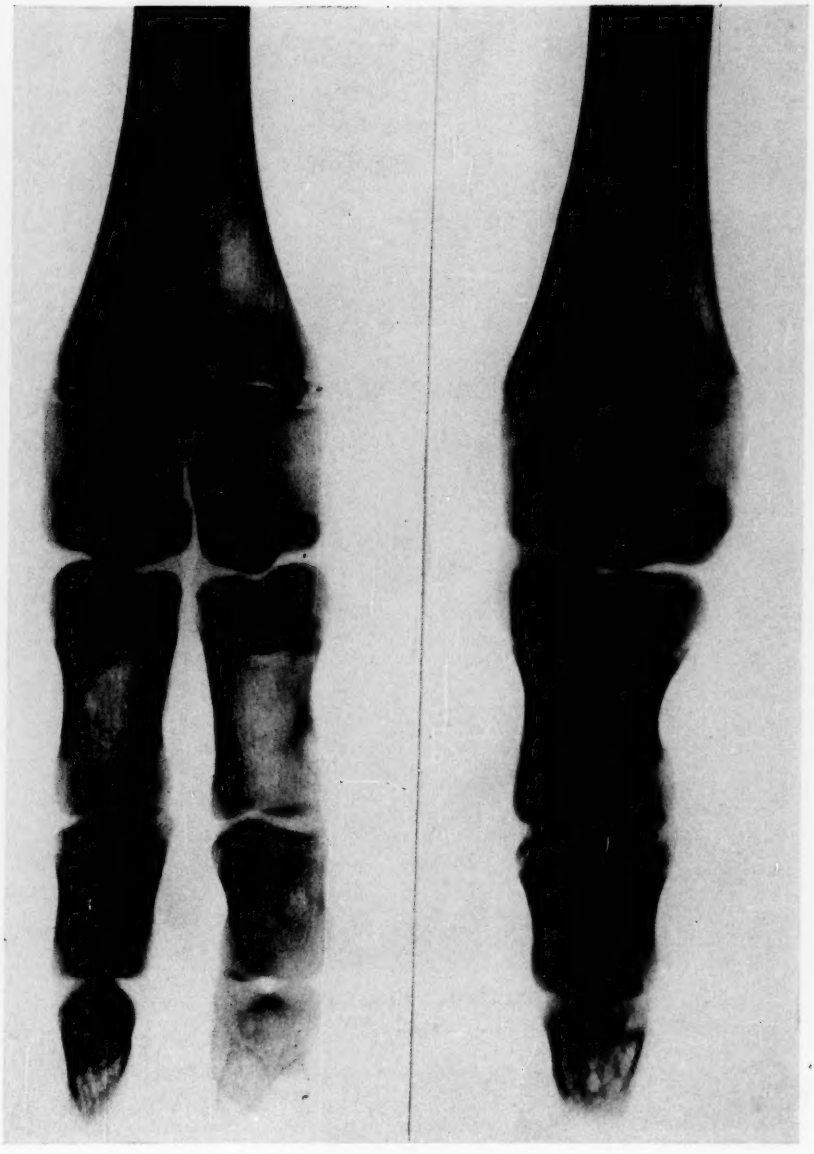
The *superficial digital flexor* did not divide into two branches at the level of the fetlock joint, but it did receive a reinforcing band from the suspensory ligament. It divided into branches which were inserted into the proximal border of the first phalanx. Normally the two branches are inserted into the volar surfaces of the second phalanges. The *deep digital flexor* did not divide at the fetlock joint but was inserted into the volar aspect of the third phalanx, similar to the normal condition.



SKELETAL DETAILS

Figure13

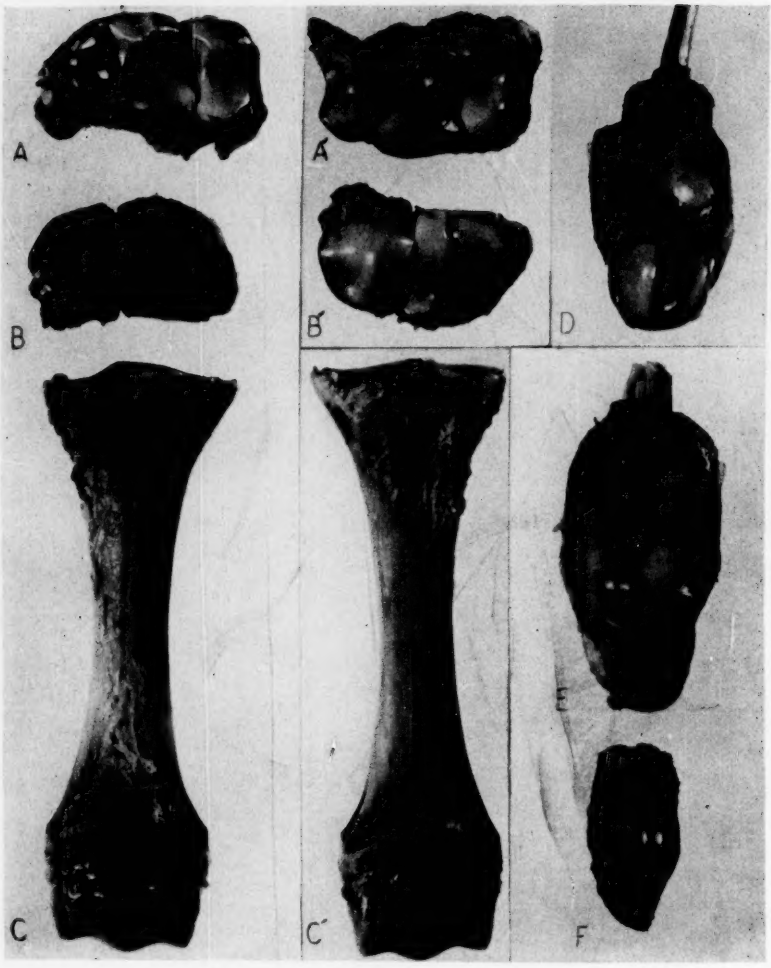
The syndactylous right forelimb is shown on the left, in comparison with the normal cloven right hindlimb of the same individual. Observation of these "mule foot" cattle indicates that the disadvantages of this variation far outweigh any advantages.



X-RAY DETAILS

Figure 14

Photographs of the two feet and shanks shown in Figure 13. The X-rays were taken from the dorsal surface of the limbs.



SYNDACTYLOUS RIGHT FORELIMB

Figure 15

Views of the dissected syndactylous, right forelimb from the bull calf whose pedigree is shown in Figure 16. *A-A'*—Distal and proximal articular surfaces of the proximal row of carpal bones, showing partial fusion of ulnar and intermediate carpal bones. *B-B'*—Distal and proximal articular surfaces of distal row of carpal bones. *C-C'*—Dorsal and volar surfaces of the metacarpus. *D*—Upper surface shown is the proximal articular surface of the second phalanx, lower surface is the distal articular surface of the first phalanx. *E*—Proximal surface of first phalanx showing proximal sesamoid bones (top structure) fused into one structure. *F*—Articular surface of third phalanx showing distal sesamoids (top structure) partially fused.

The *suspensory ligament* divided into three branches at the distal end of the metacarpus. The medial and lateral branches were inserted on the proximal sesamoid bones, the first phalanx, and the distal end of the metacarpus. A small slip was given off from the lateral side of the suspensory ligament above the trifurcation similar to that which joins the superficial flexor in the normal foreleg, but it ended blindly in the fascia. There was no similar slip on the medial side. According to Sisson and Grossman,[7] normally "The middle band passes through the groove between the two divisions of the distal end of the metacarpus . . ." In this specimen, (Figure 15, *C* and *C'*) there was no groove; consequently, there was little similarity to the normal in the attachment of the middle band. The middle band of the suspensory ligament did not trifurcate, but ended on the proximal sesamoid bones and sent off a slip which joined the superficial flexor at about the level of the fetlock joint. There were no connections between the suspensory ligament and the extensor tendons.

The *volar common digital* artery divided above the fetlock, superficial to the tendons, and the two branches, medial and lateral, passed downward across the abaxial surface of the sesamoid bones and continued in the digit as medial and lateral digital arteries similar to the anatomy of these parts in the horse. The medial and lateral digital arteries gave off the dorsal artery of the first phalanx, the dorsal artery of the second phalanx, and terminated as the dorsal artery of the third phalanx. The *dorsal metacarpal* artery decreased in size until it terminated at the distal end of the metacarpus. The *medial* and *lateral deep volar metacarpal* arteries ended in the fascia and skin just above the fetlock joint.

The digital nerves were terminal branches of the median nerve accompanying the digital arteries.

The ulnar and intermediate carpal bones were found to be fused posteriorly, as viewed from the distal surface, Figure 15*A*. This fusion was the most proximal deviation from the normal anatomy in the osseous system. The fused second and third carpals, the fourth carpal, the accessory carpal, the small metacarpal and the proximal articular surface of the metacarpus appeared normal. The dorsal surface of the metacarpus, Figure 15*C*, had only a faint vertical vascular groove. The distal end of the metacarpus was not divided by a sagittal notch but presented an articular surface very similar to that of a horse, especially from the dorsal view. The first and second phalanges were solidly fused, and were larger in diameter than the corresponding phalanges in each digit of the rear foot of the same animal. The third phalanx was slightly larger than either of the third phalanges of the rear foot and somewhat more symmetrical. In its symmetry, the third phalanx resembled that of a horse or mule, but it was much narrower and more pointed. Normally each digit has two proximal sesamoids and one distal sesamoid. In this specimen there was one proximal sesamoid, solidly fused in cartilaginous material, with three distinct centers of ossification, (Figure 15*E*), and two distal sesamoids only partially fused, (Figure 15*F*), with the medial sesamoid approximately three times as large as the lateral sesamoid.

Six other syndactylous calves have been observed by the authors, four of which have been purchased for observation and breeding tests. Also, reliable descriptions have been obtained from the owners of nine more syndactylous calves, making a total of 17 considered thus far. Among these animals considerable variation occurs.

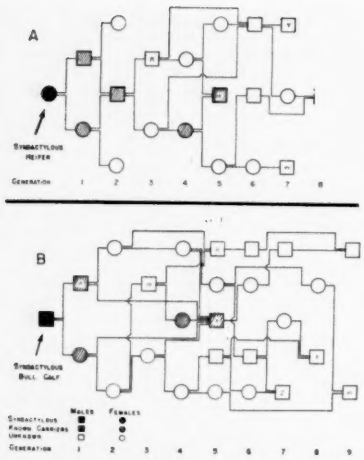
There were cases in which only one front foot was syndactylous, cases in which three of the feet were syndactylous, and cases in which all four feet were syndactylous. In addition to the variatiton in the number of feet affected, there was also variation in the apparent degree of fusion. In most cases, there was no evident line of fusion in the affected front feet, they being similar to those of the calf which was dissected. The affected feet, however, often were only partly fused, some being solid but with the line of fusion quite apparent, while others were cleft anteriorly, but with a solid sole. Those which have been under close observation have been found to be less thrifty than their normal pen mates, but the numbers are too small to warrant general conclusions.

The rear feet on the calves which have only the forelegs syndactylous have been found to have the following rather uniform deviation from normal. Proximal to the cleft in the foot and at about the level of the first and second phalanges the anterior groove between the digits is more pronounced and deeper than in normal animals.

Inheritance

Of the 17 cases of syndactylous calves observed by or reported to the authors, 15 were inbred Holstein-Friesian calves from normal parents tracing on both sides of the pedigree to one side (hereafter referred to as Sire *X*—see Figure 16). Syndactylous calves were found among both males and females. The simplest explanation for such a pattern of occurrence was that the character was inherited as a single autosomal recessive and that Sire *X* was heterozygous. Accurate records of a unique series of matings were available to test this hypothesis.

Sire *X* produced a total of 287 offspring during his service in the herd



PEDIGREES OF TWO SYNDACTYLOUS HOLSTEIN CALVES

Figure 16

The alphabetical symbols on some of the bulls refer to the text and Table I. *A*—Pedigree of the first syndactylous heifer reported to the authors. Five generations of normal, unsuspected heterozygotes separate this syndactylous heifer from the original sire in this strain of cattle, Sire *X*. *B*—Pedigrees of the syndactylous bull calf whose limbs were used for a detailed anatomical description of the characteristic. The combination of information from these two pedigrees led to the further investigation of Sire *X*. The frequency of the "mule foot" gene is low in the Holstein breed, but the recent popularity of males who may be carriers may have increased this frequency considerably.

where he was used. Of this number, 131 progeny were known to be from outcross matings, 40 were from matings to his own daughters, and 19 were from matings to his own granddaughters. Each of his own daughters to whom he was mated had a coefficient of relationship to him of 50 percent. Of his granddaughters to whom he was mated, the coefficient of relationship to him were as follows: two with 50 percent, two with 37.5 percent, and 15 with 25 percent. Detailed information was not obtained for the remaining 96 offspring, but the owners reported that all 96 were from outcross matings and none was observed to have syndactylous feet.

The 131 offspring from the outcross matings were all reported to be normal as would be expected if it were assumed that the character is inherited as a single recessive, that Sire *X* was heterozygous, and that the gene had a frequency approaching zero in the breed, or among the unrelated animals in the herd to which Sire *X* was mated. Five single-toed calves occurred among the 40 progeny from the sire by daughter backcross, a perfect fit to the expected 7:1 ratio. All of the 19 calves from the sire by granddaughter crosses were normal, although seven percent, or 1.37 calves were expected to be single-toed, when adjustments had been made for different degrees of relationship. These findings strongly support the hypothesis that this characteristic is inherited as a single recessive. Final conclusions concerning the mode of inheritance should not be made until some single-toed individuals have been mated together to see whether the character breeds true.

The two cases of syndactylism which were exceptions to the above observations were from an altogether different strain of Holsteins. In the community where these two cases occurred there were few cattle which

TABLE I.—Distribution of Progeny from Matings Between Descendants of Sire *X*. The progeny are arranged according to relationships of their respective sire and dam to Sire *X*.

Male Used	Coef. of Relationship	Females						Expected Syndactylous Calves *	Observed Syndactylous Calves
		75	62.5	50	37.5	25	Total		
A	62.5			5	1		6	0.72	1
B	50			1			1	0.12	0
C	50			2			2	0.25	0
D	50			1	2		3	0.31	0
E	50			7			7	0.88	0
F	50	1	1	29	3	13	47	5.06	0
G	50			30		1	31	3.81	0
H	25			3			3	0.38	0
J	25	3		56	1	1	61	7.72	0
K	25			6		1	7	0.81	0
L	25			1	1		2	0.22	0
Total		4	1	141	8	16	170		1

*The number of syndactylous calves expected from each bull was based on the hypothesis that each bull was heterozygous, and the relationship of the dams to Sire *X* was a measure of the frequency of the recessive gene in each group of dams. The comparison between the expected and observed then could be used in the case of each bull to test this hypothesis. No adjustment was made for removal of homozygous recessives in the few cases where such removal could have influenced the gene frequency.

were related to the strain in which the majority of the syndactylous calves occurred. In only one of the two cases could the ancestry be traced with a fair degree of accuracy, and investigation of the ancestors did not reveal any other instances of syndactylism. These two cases are comparable to those reported in the past in which the frequency of occurrence of the character was so low that they were considered merely freaks and forgotten.

Additional information for checking the hypothesis of a single recessive gene was provided by data from 170 progeny which resulted from matings between the descendants of Sire *X*.

For two reasons it was not considered justifiable to estimate the probable occurrence of single-toed individuals among the progeny on the basis of random mating between the descendants of Sire *X*, even after adjusting such estimates for relationship of each of the two parents to him. First, it was the consistent policy within each herd where syndactylous calves occurred to remove the syndactylous individuals from the breeding herd disturbing the random distribution of the gene; and second, the number of progeny per bull varied from 6 to 61 offspring. The number of individuals removed from the breeding herd because they were syndactylous, and consequently postulated to be homozygous for the recessive gene, was so small that it would have little effect. However, the inequality in number of offspring would have tended to bias the results considerably since any one normal male descendant of Sire *X* could have been only one of two genotypes, either heterozygous or homozygous for the dominant allelomorph. If heterozygous and the sire of a large number of offspring, there would have been many more syndactylous calves in the herd than would have been expected from random mating; and, if homozygous dominant, there would have been many fewer syndactylous calves than would have been expected from random mating. Therefore, each descendant male was considered separately as shown in Table I.

If the bulls *F*, *G* and *J*, Table I, had been heterozygous, each would have been expected to have several syndactylous calves among his offspring from the matings which were made. No case of syndactylism was reported among the 139 offspring of these three bulls. This evidence was not necessarily conclusive but was a strong indication that these bulls were homozygous for the dominant allelomorph or non-carriers. The probability of a breeder selecting three bulls and getting three non-carriers with the relationship of 50 percent, 50 percent, and 25 percent to a known heterozygote is one to five and one-third, which is not too improbable. Since these three bulls were the ones used most extensively, however, the breeders were fortunate in selecting three apparent non-carriers against such odds. Of the other bulls, *A* was the only one to have a syndactylous calf, and he had one among six. One other son, Sire *K*, was also found to have transmitted the gene. Sire *K* had one son which was used as a foundation male for an inbreeding program in one herd, resulting in several syndactylous calves.

The results obtained from the matings between the descendants of Sire *X* fit well into the hypothesis that the character is inherited as a single recessive. Bulls *A* and *K* (Table I) were proved to be carriers by results from their own offspring or offspring in subsequent generations; three bulls, *F*, *G*, and *J*, had a high probability of being non-carriers; and the other six bulls had insufficient numbers of offspring to warrant conclusions. These results might fit another more complicated pattern of inheritance, but they do not contradict the simple explanation developed.

Discussion

A number of men closely associated with Holstein-Friesian as well as with other breeds of cattle were questioned concerning the frequency of occurrence of syndactylism, single-toes, or "mule-feet" in cattle. None had ever observed this characteristic prior to the instances reported in this paper. These observations indicate a low frequency of the gene, especially when coupled with the facts that two known carriers used in different herds produced no syndactylous calves in approximately 400 matings with unrelated cows. One of the bulls, proved to be a carrier of the factor for single-toe, was outstanding in transmitting both production and type, and has been responsible for establishing somewhat of a sub-family or strain within the breed. As a result of his recognized merit, well over 100 sons have been used as herd sires throughout the country. One-half of his sons should be heterozygous or carriers. It is probable, therefore, that there will be observed in the future an increase in the occurrence of syndactylous calves.

Several persons have raised questions concerning the possible value of syndactylism, including a suggestion by one breeder that with a solid hoof there would be less chance for foot-rot to develop. In order to answer accurately such questions the animals purchased have been carefully observed.

Although the oldest animal of the four purchased thus far for observation and breeding tests is only 13-months old, there has been observed a marked tendency for the feet to become sore, and the animals to be unthrifty. One animal with one normal and one syndactylous forefoot has been observed to favor the syndactylous foot consistently. The other afflicted animals are not so agile as their normal-footed pen mates, the difference being especially noticeable on concrete floors and also quite apparent in the pasture. One of these animals has three feet syndactylous and the fourth with a solid sole, but cleft anteriorly. This latter foot has been especially susceptible to infection, as might be expected since it has

a pocket in which to accumulate debris. It seems now that there is no advantage in syndactylism in calves, and that there are several disadvantages.

The evolutionary significance of syndactylism is a subject for speculation. It should be pointed out, however, that in distinction from the horse, this character is definitely a fusion of two digits distal to the normal termination of fusion between the third and fourth metacarpals. It is not an enlargement of one digit accompanied by a deterioration of the other as occurred in the evolutionary development of the modern horse.

As in swine, humans, and other animals affected with either syndactylism or polydactylism, the forelimbs in the affected cattle show more fusion than the hindlimbs. This fits in with the growth gradient theory first proposed by Child[2] and well discussed by Thompson.[8]

Difficulties of Eliminating Syndactylous Gene

As with other characters inherited as recessives, the breeding methods for eliminating the syndactylous gene or preventing its dissemination through a herd or breed are not simple. The recessive phenotypes, i.e. the syndactylous calves, can be identified without difficulty and eliminated, but such culling has only a limited effect in decreasing the frequency of a gene which has a low initial frequency. The heterozygotes, or carriers, would need to be identified and removed in order to eliminate the gene from a herd. Carriers can be identified positively, and separated from non-carriers or suspected carriers, by one criterion only, i.e. by siring or giving birth to a syndactylous calf. Since the birth of a syndactylous calf requires both parents to be either syndactylous or heterozygous, the location of carrier females can be accomplished only by mating the suspected females to a syndactylous or known carrier male. Mating to a syndactylous male would identify on the average one half of the carrier females in one calf crop, but unless the entire calf crop were slaughtered the net change in frequency of the gene in the herd would be increased. Since a breeder usually cannot afford to sacrifice a complete calf crop it is impractical for him to try to test-mate his herd in this manner. Matings made before the character was first identified, however, may be used as tools for locating known carriers. Young males can be tested also by mating them to known carrier females before breeding them to the entire herd. About the only practical step a breeder can take toward eliminating the gene is to stop using any known carrier males or to resort to completely unrelated males. By correct selection of the males, the frequency of the gene can be halved each generation.

The breeders who made available the information presented in this paper are to be commended, since through their cooperation it has been possible to determine the mode of inheritance of syndactylism in cattle. The next time a breeder has a mule-footed calf born in his herd he will be in a position to recognize it immediately as an inherited characteristic instead of just a freak, and to make the changes in his breeding program necessary to eliminate it. Thus, when a characteristic first appears, the cooperation of a few breeders makes it possible for a large number of breeders to avoid breeding trouble in the future.

Summary

A number of cases of syndactylism were found in Holstein-Friesian cattle, one of which was dissected and is described in detail. The mode of inheritance of the character was found to follow the pattern of a single, autosomal recessive. Observations indicated that the disadvantages of the character far outweighed its possible advantages.

The observations indicate a low frequency of this gene in the present cattle population, but recent distribution of carrier males may have increased the frequency considerably.

Author's Note: Since the manuscript for the preceding paper was submitted for publication, some data published in India has come to the attention of the authors. The occurrence of syndactylous calves in Hariana cattle, a breed of Zebu cattle used for milk, draft and beef was reported by S. Singh and R. K. Tandon (*Indian Jour. Vet. Sci. and Anim. Husb.* 12:61. 1942). Using some additional data, as well as the data originally reported, S. Singh and P. Bhattacharya (*Indian Jour. Vet. Sci. and Anim. Husb.* 19:153-159. 1949) reported that the characteristic apparently was inherited as a single autosomal gene. Their findings in the Hariana breed of cattle are in agreement with the conclusions herein reported for Holstein cattle. Examples of apparently identical genes acting on different breeds of cattle in widely separated countries are of interest.—F. E. Eldridge.

Literature Cited

1. Bateson, W. Materials for the Study of Variation. Macmillan & Co., New York. 1894.

2. Child, C. M. *Biol. Bul.* 39:147-187. 1920.

3. Petlefsen, J. A., and W. J. Carmichael. *Jour. Agr. Res.* 20:595-604. 1921.

4. Gates, R. R. Human Genetics. Macmillan Co., New York. 1946.

5. Kale, C. G. *Indian Vet. Jour.* 24(5): 350. 1948.

6. Ross, O. B., *et al. Jour. Anim. Sci.* 3: 406-414. 1944.

7. Sisson, S., and J. D. Grossman. The Anatomy of the Domestic Animals. W. B. Saunders Co., Philadelphia, 3rd Ed. Pp. 250, 345. 1938.

8. Thompson, D. W. On Growth and Form. Macmillan Co., New York. P. 283. 1942.

(*Received October 27, 1950*)

CYTOLOGICAL EFFECTS OF SOME MORE ANTIBIOTICS

G. B. Wilson and C. C. Bowen*

In a previous publication[1,2] the senior author reported briefly on the cytological and other effects of seven antibiotics on *Allium* root tips. These seven were: Penicillin G, Streptomycin, Neomycin, Circulin, Endomycin, Actidione and Streptothricin. In general, Penicillin G and Endomycin had little measurable effect on mitosis but the latter did appear to have an effect on growth pattern in that it stimulated the production of secondary roots, a fact that has since been confirmed with a much purer sample of the drug. Neomycin and Circulin were also without very striking effects on mitosis but were very highly, and almost immediately toxic. The remaining three readily induced mitotic breakdown of the type generally, though perhaps unfortunately, referred to as "C-mitosis." In all three cases recovery from doses capable of inducing cytological aberrations, so far has not been obtained.

The present paper is concerned with the effects of four medically important antibiotics: Aureomycin, Terramycin, Streptomycin and Chloromycetin.

Methods and Observations

Materials and methods were essentially as described previously. Onion bulbs were rooted in aerated tap water and the roots checked for "normality" of division. Treatment was in distilled water solutions of the various antibiotics. Concentrations used were 25, 50, 100, 150, 200, and 300 parts per million except in the case of Chloromycetin where additional concentrations of 400, 600, 800 and 1000 ppm were tried. Collections were made at six and 12 hours after which the remaining roots were thoroughly rinsed and brought back to tap water which was changed after four, 12 and 24 hours. Further collections were made at intervals up to 36 hours in order to check for recovery. Fixation was in three parts absolute alcohol and one part glacial acetic acid and preparations were made by the Feulgen "squash" technique.

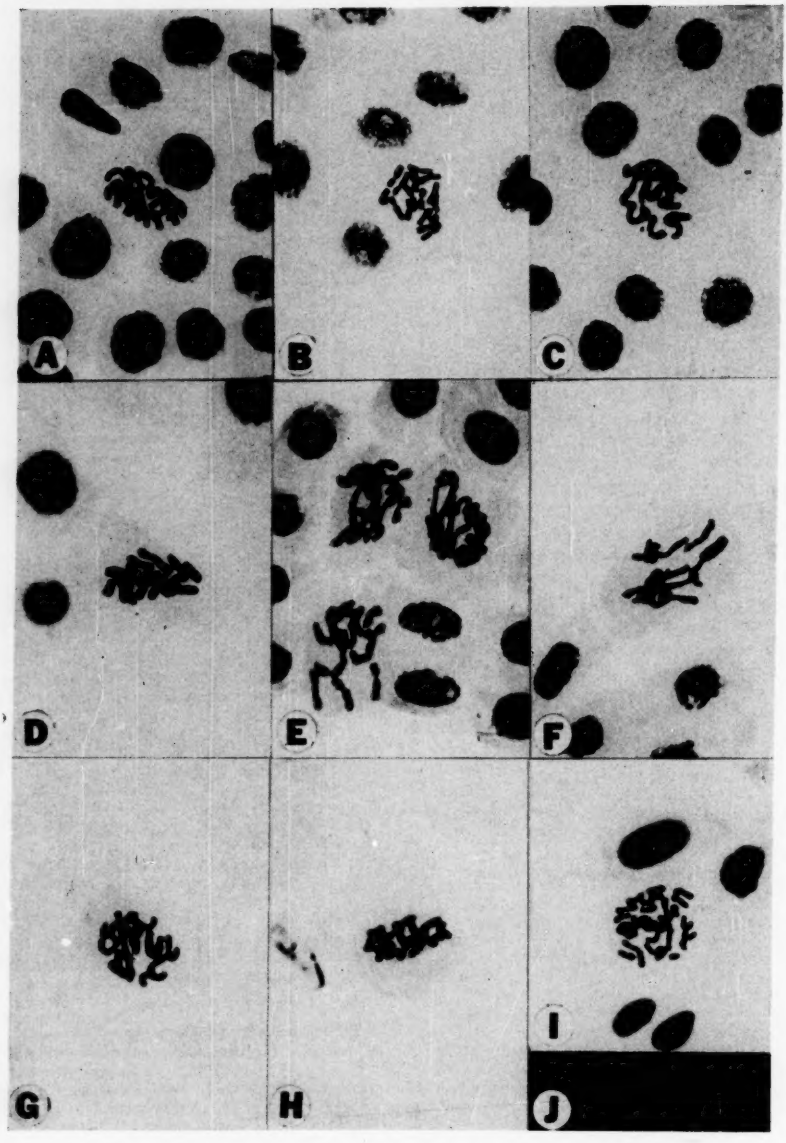
1. *Aureomycin*

Aureomycin is a product of *Streptomyces aureofaciens*. The sample used was the hydrochloride bearing the Lederle Laboratory Division, American Cyanamid Company's number 7-6723. Less than twelve hours exposure to concentrations varying from 50 to 100 ppm produced mitotic deviations similar to those previously described for Acti-dione (Figure 17*A* and *B*). These include over-contraction of the chromosomes, varying degrees of spindle malfunction and apparent delay in nuclear membrane dissolution. Some "reductional groupings" were noted but whether these were more frequent than in controls has not yet been determined. Concentrations of 150 ppm and above have strong toxic effects within six hours as indicated by clumping and stickiness in mitotic figures and by a heavy opaque staining of the energic nuclei many of which are amoeboid in shape. Exposure to 100 ppm and up resulted in subsequent death of the roots indicated by loss of turgor in the region of elongation. A marked yellowing of the meristematic region suggests accumulation of the drug. Recovery was not obtained after 12 hours treatment with any concentration sufficient to produce cytological effects. After 36 hours of attempted recovery, the majority of cells in the apparently dead roots showed a uniform, faint Feulgen positive reaction throughout the protoplast with nuclear outlines frequently becoming indiscernable even under the phase microscope.

2. *Terramycin*

Terramycin is produced by *Streptomyces rimosus* and has been used as the hydrochloride bearing the Chas. Pfizer & Company control number WBQ507422. Both cytologically and morphologically the effects appear to be very

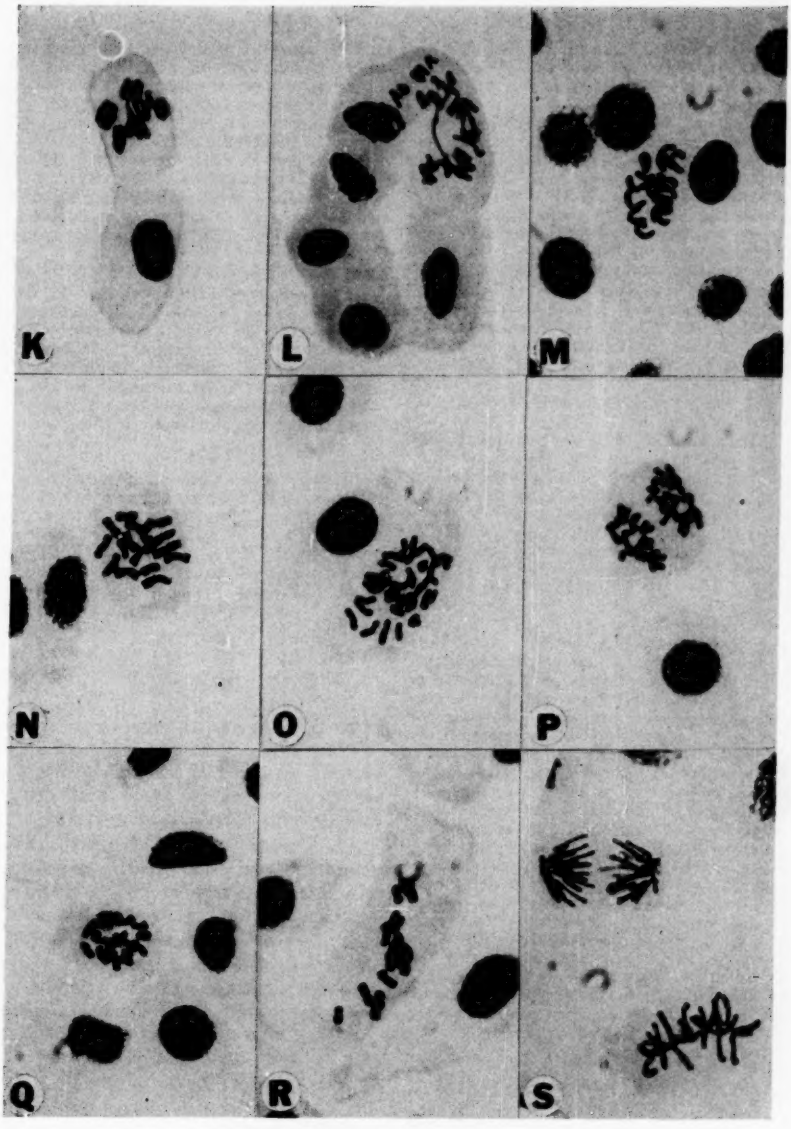
*Associate Professor and Graduate Assistant respectively, Department of Botany and Plant Pathology, Michigan State College, East Lansing, Michigan. The authors wish to express their gratitude to: The Lederle Laboratory; Chs. Pfizer & Company; the Upjohn Company and Parke Davis & Company who supplied Aureomycin, Terramycin, Streptomycin and Chloromycetin respectively. We also wish to thank Mr. P. G. Coleman, Michigan State College Experiment Station Photographer for preparation of the plates. Financial assistance was provided by the All College Research Committee and the Experiment Station. Contribution 51-8 of the Department of Botany and Plant Pathology, Michigan State College.



EFFECT OF AUREOMYCIN, TERRAMYCIN AND STREPTOMYCIN

Figure 17

A-B—Typical effects of 150 ppm (parts per million) of Aureomycin for 12 hours. *C-D*—Typical effects of Terramycin on prophase and metaphase. 200 ppm for six hours. *E-F*—Toxicity symtoms and reversion. Terramycin, 100 ppm for six hours. *G-I*—Effects of prophase, metaphase and anaphase of 50 ppm Streptomycin for 12 hours. The over-contraction is typical, but lack of spindle organization is found in a minority of cells after this treatment. *J*—Shows magnification of all figures. Each division of the scale represents 10 microns.



CONTRACTION AND STICKINESS

Figure 18

K-L—Stickiness and reversion after treatment with 100 ppm Streptomycin for 12 hours. *M-P*—Effects of 1000 ppm Chloromycetin for six hours. The anaphase in *P* is more or less normally organized but has very much contracted chromosomes. *Q-R*—Effects of 1000 ppm Chloromycetin for 12 hours. *S*—Normal metaphase and anaphase after 21 hours "recovery" from treatment with Chloromycetin for 12 hours.

similar to those of Aureomycin (Figure 17*C* and *D*) except that the meristem did not show any coloration despite the fact that this drug is as deep a yellow in solution as Aureomycin is an orange. Treatment with 100 ppm produced marked effects on mitosis within six hours and general necrosis within 12. Lower concentrations had no appreciable effect within the time range used. There appears to be somewhat less chromosome contraction accompanying spindle disruption (Figure 17*E*) than is characteristic of Aureomycin and Actidione. Occasional figures were noted in which metaphase chromosomes appear to be reverting to the "telophase" condition *in situ* (Figure 17*F*), a condition much more common after treatment with Streptomycin. So far no recovery of affected roots has been noted.

3. *Streptomycin*

This antibiotic is a product of *Streptomyces griseus* and, in the present instance, has been used as the sulphate bearing the Upjohn Company's number Z-5413. In general the results were found to be as previously described. Chromosome aberrations involving over-contraction and spindle breakdown were induced by doses of 50 to 100 ppm (Figure 17*G—I*). At the latter dose most dividing figures showed various degrees of stickiness and clumping (Figure 18*K* and *L*). The majority of prometaphases and later stages have a tendency to revert to the energic stage either as individual chromosomes or in small groups (Figure 18*K*). Except for the extreme contraction of the chromosomes at metaphase there appeared to be less disruption of the "C-mitotic" type than found after treatments with any of the other antibiotics included in this study. However, all affected roots showed some spindle disruption. At toxic levels both cytological and morphological changes differ somewhat from those produced by other treatments. Early prophase figures appear to be more or less normal, energic nuclei become fibroid rather than more or less homogeneous in appearance and the majority of later stages of division have a tendency to revert to the energic condition rather than becoming oily appearing clumps. Morphologically the roots remained normal in appearance and without any notable loss in turgor but became extremely brittle. Again no recovery of affected roots has so far been obtained.

4. *Chloromycetin*

Chloromycetin, an antibiotic produced by *Streptomyces venezuelae*, was used in the synthetic form manufactured by Parke Davis and Company. The control number of the sample used was 137954. At doses below 1,000 ppm (which appears to be about the solubility limit) little or no indication of either cytological effect or toxicity was noted. After six hours exposure to 1,000 ppm, however, most dividing cells showed typical "C-mitotic" like aberrations (Figure 18*M—P*). By 12 hours only a few division figures were found and all of these were markedly affected (Figure 18*Q* and *R*). Unlike roots treated with the other antibiotics, most Chloromycetin treated material recovered and continued growth and division (Figure 18*S*). At the end of 12 hours recovery there were abundant apparently normal division figures with, so far as we were able to determine, no polyploidy or other abnormalities.

General Observations

All four of the antibiotics discussed above are capable of inducing mitotic aberrations of a type superficially similar to those generally grouped together under the term "C-Mitosis." With the exception of Chloromycetin, the cytological and toxicity thresholds are so close together that recovery of altered cells is unlikely (see Table I). Even Chloromycetin is a division suppressor so that actually relatively few cells undergo aberrant mitosis and recovered cells, therefore, would be expected to be primarily normal. None of these drugs are likely, therefore, to induce polyploidy or other permanent changes in great numbers. In all cases some "reductional" or "segregational" type groupings were found but it is doubtful if their incidence is substantially higher than in controls.

Discussion

Even a superficial survey of the now very extensive literature on the cytological and genetical effects of chemicals is sufficient to establish some fundamental, and perhaps even alarming, facts:

1. Several organic chemicals have definitely been established as capable of producing recoverable polyploidy; e.g., colchicine[2,8] and cyclochlorohexane.[7,9]

2. Many other chemicals, both organic and inorganic, have been shown to produce mitotic breakdown precursory to polyploidy; e.g., inorganic phosphates,[4] salts of nucleic acid,[6] several antibiotics,[12] naphthelene acetic acid,[3] D.D.T.,[11] and ethylene glycol.[3]

TABLE I.—A summary of the Cytological and Morphological effects of several antibiotics.

Substance	Cyt. effect threshold	Toxicity threshold	Recovery at Cyt. effect threshold
Aureomycin	50-100ppm	150ppm	none
Streptomycin	50-100ppm	100ppm	none
Terramycin	100ppm	100-150ppm	none
Chloromycetin	100ppm	>1000ppm	±80 per cent

3. Some chemicals appear to increase the normal incidence of mitotic deviations precursory to chromosome reduction and/or genetical segregation; e.g., sodium nucleate,[5] colchicine,[1] Actidione[12] and cyclochlorohexane.[13]

4. An increasingly large number of chemicals appear to be capable of inducing chromosome fragmentation and other rearrangements similar to those produced by radiation; e.g., acenapthene, benzedrine, toluidine blue[3] and pyrogallol.[10].

5. A few chemicals appear to be able to induce gene mutations in the strict sense; e.g., the mustards and several of the carbamates including urethane.[3] It seems likely that many more will be added, in due course, to those already known.

These facts mean simply that the chemical environment of organisms is potentially capable of inducing significant changes in their genetic make-up. It therefore follows that the widespread use of many of these potential or actual chemical mutagens in medicine and agriculture is not without genetic significance and deserves at least as much investigation as is accorded the genetic consequences of the release of atomic energy.

Our own investigations have led us to take a more sanguine view of the potentialities than the foregoing would indicate. Experience with the antibiotics reported on herein as well as with other chemicals of like nature (the cytological effects of which we have studied) incline us to the belief that, in most cases, little of the potential for genetic or cytological change is likely to be realized. This is partly due to the fact that the mutagenic and lethal thresholds are generally close together. Furthermore, selection at the level of cellular reproduction is likely to operate against the perpetuation of altered nuclei. Nonetheless a high potential for change exists. How much of it is likely to be translated into actuality can only be determined by long and tedious experimentation.

Literature Cited

1. Allen, N. S., G. B. Wilson, and S. Powell. *Jour. Hered.* 41:227-231. 1950.
2. Brues, A. M., and A. Cohen. *Biochem. Jour.* 30:1363-1368. 1936.
3. Buzzati-Traverso, A. (Ed.) *Pub. della Stazione Zool. di Napoli.* 22 (suppl.). 1950.
4. Galinsky, I. *Jour. Hered.* 40:289-295. 1949.
5. Huskins, C. L. *Jour. Hered.* 39:311-325. 1948.
6. Kodani, M. *Jour. Hered.* 39:327-335. 1948.
7. Kostov, D. *Nature.* 162:845-846. 1948.
8. Nebel, B., and M. L. Ruttle. *Jour. Hered.* 29:2-9. 1938.
9. Nybom, N., and B. Knutsson. *Hereditas.* 33:220-234. 1947.
10. Tjio, J. H. *An. Aula Dei.* 2:187-194. 1951.
11. Vagrama, A. *Hereditas* 33:191-219. 1947.
12. Wilson, G. B. *Jour. Hered.* 41:227-231. 1950.
13. ———————, M. E. Hawthorne, and Te May Tsou. *Jour. Hered.* 42: 183-189. 1951.

(*Received July 5, 1951*)

◆

THREE IN ONE: PHYSICAL ANTHROPOLOGY, GENETICS, STATISTICS

PROF. Boyd's interest in applying discoveries in genetics to the problems of physical anthropology is well known. The present book* gives the results of his efforts in this direction, together with a summary of human genetics in general. This division in subject matter is foreshadowed in the title. In the text the division has not been so successfully followed and the result is confusing. Actually, there is a book on genetics partially hidden within a book on physical anthropology (or it may be *vice versa*), with a third book (on statistics) thrown in as an appendix.

At the beginning of the book (p. 3) Prof. Boyd conceived his task as follows: "In this book we shall be concerned with the study of physical anthropology." Later, when he got around to writing the preface, he said (p. xvi), "The chief effort in the present writing has been to acquaint the reader with the im-

*Boyd, William C. *Genetics and the Races of Man.* pp. xvii + 453. Price $6.00. Little, Brown and Co., Boston. 1950.

portant results of the newer [genetical] work, especially as they might apply to man." If he had followed up this hindsight by cutting out or modifying the chapters (1, 7, 9, 12 and 13) which present his version of physical anthropology, he would have had a more coherent book and one which both physical anthropologists and geneticists could praise.

The remarkable advances in the science of genetics have been derived in but small part from observations on man, and especially on man in a normal or a healthy state of being. The truth of this assertion is underscored by the fact that the American Society for Human Genetics is only three years old. In spite of this slow progress, physical anthropologists have continued to regard this field as one of high promise. Witness that many original data on the blood groups and types that have been published in the *American Journal of Physical Anthropology*. Furthermore, Prof. Boyd's outstanding contributions on this subject are recognized in the form of membership on the editorial board of that journal.

"The Record" of Physical Anthropology

In contrast to the yet restricted knowledge of human heredity, physical anthropology has accumulated during the past century or more a vast record of man's physical variations. Working in the tradition of zoology, physical anthropologists have used this anatomical knowledge to classify the peoples of the world. The results have varied somewhat, of course, depending on the characters used. Yet the races thus established years ago have provided the framework wherein all subsequent study of man has been organized.

Consider, for example, the blood group data. In going about their work, serologists have carefully segregated peoples according to the traditional racial groupings. In a town like Nome, Alaska, they would be concerned especially to separate Eskimos from Whites. Indeed, it is considered unscientific to ignore such phenotypic indications. Prof. Boyd himself, some years ago, compiled blood group data in this racial framework for all the peoples of the world.

In view of this history, it can be perceived that it is no easy matter to divorce the accumulated data from their conceptual framework. Thus, when Prof. Boyd sets out in the present book to classify man on the basis of the existing blood group data, he emerges with a racial scheme that is scarcely different from the old. And why should he not get this result? Although the genetic mechanisms of most phenotypic characters have not been worked out, it would be strange if the genes involved, like those for the blood groups, did not reflect the past history of mankind.

The Genetic Approach

An attempt to classify man on the basis of genes is a legitimate procedure and an important part of the application of genetic knowledge to man. As such it can stand on its own feet and be sure of a welcome from physical anthropologists. Unfortunately, however, Prof. Boyd has felt that he must bolster this procedure by disparaging the ideas of certain physical anthropologists. Usually these are old, discarded ideas that served their purpose and are now of only historical interest. For example, he takes Dixon's experiment in classification published in 1923, deplores the concepts behind it, and by using the present tense gives the impression that the idea is still current, although it never was widely accepted and often has been deplored.

Another way in which Prof. Boyd disparages physical anthropologists is by insinuating that their experience in observing mankind gives only ridiculous and hence valueless results. The following is an example of this sort of argument:

> That not a few anthropologists as well as laymen were once convinced that they could identify race by a mere inspection of the individual is rather well shown by Sir Arthur Keith's remarks: "A Negro or a Chinese, a Hindu or an American Indian meets us in the street, and, before he has passed, we have assessed his racial characters and made our diagnosis. We can never hope to obtain, by the use of instruments, indices, and angles, the delicacy and precision of racial diagnosis reached by the tutored and experienced eye. Travelers and explorers may become so skilled that in a cosmopolitan crowd in an eastern bazaar they will recognize the most delicate shades of race."
>
> How well Sir Arthur could have sorted his individuals into races if they had filed past him clean-shaven and naked, we do not know. . . . (p. 6-7)

I think the reader will agree that in this case a quicker and more useful distinction would result from inspection of external characters than from the determination of blood groups. The practiced eye is still helpful in science.

The Question of "Objectivity"

Prof. Boyd either directly or by implication also makes undesirable comparisons between scientific procedures. Thus, the genetic classification of races and the genetic approach to the study of man are described as "more objective, and better founded scientifically" (p. 274) and more intelligent and more profitable (p. 349), than the "old fashioned" classifications and approaches, respectively. Who knows but that future students may smile at Prof. Boyd's *naivete*!

These criticisms apply almost exclusively to the five chapters on so-called physical anthropology enumerated above, which bear the following titles: Possible Approaches to a

(*Continued on page 260*)

STUDIES ON SEMI-LETHALISM IN THE MALE LORDOTIC GUPPY

Lebistes reticulatus

HAROLD L. ROSENTHAL*
Philadelphia Aquarium Society, Philadelphia, Pa.

IT is well known that viable sperm may be retained in the ovary of female viviparous cyprinodonts for long periods of time. Van Oordt[4] states that in *Xiphophorus*, sperm from a single copulation may bring about fertilization of a group of ova each month for ten months. The recent report of Clark[1] is of considerable interest. She has shown by a smear technique that a single insemination resulting from normal copulations does not always produce broods in *Platypoccilus* and *Xiphophorus*. Haskins and Haskins[2] have indicated that sperm of the albino *Lebistes*, in contrast to the wild-type, rarely survives in the ovary for more than one brood unless the female is reimpregnated. Thus, the albino character is associated with a semi-lethal condition in the male.

In the course of studies on the genetics of the lordotic mutation in *Lebistes* (Rosenthal and Rosenthal[3]), it was found that mutant female fish mated with mutant male fish apparently gave birth to average numbers of young in successive broods when they were refertilized soon after parturition. In a few cases, however, refertilization of the females was omitted, and it was observed that the number of young in succeeding broods decreased significantly, indicating that spermatozoa of mutant fishes were less viable. The present work was undertaken, therefore, to study more completely the apparent semi-lethal character of the lordotic mutant.

Materials and Methods

The lordotic mutation in the guppy behaves as an autosomal, single factor Mendelian character, recessive to the normal. The recessive allele has been designated S_e and the normal wild-type S_n. Since the lordotic phenotype is markedly visible even at birth, it is possible to use the lordotic and wild-type progeny to indicate fertilization by a specific male.

All fish of the mutant strain were raised in the laboratory but wild-type male fish of known genetic purity were obtained from members of the New Jersey Aquarium Society. The aquaria, illuminated by a bank of reflectors for 12 hours daily since no natural lighting was available, were maintained at an average temperature of 22°C. The fish were fed daily a varied diet consisting of commercial dry foods, tubifex worms twice weekly and fresh or frozen daphnia in season. Conditions were optimal as evidenced by the regularity of brood production and the excellent physical state of the animals throughout the experimental period.

Virgin females of the lordotic strain were obtained by the method previously described by Rosenthal and Rosenthal[3]. Fully mature females, four to eight months of age, were placed in one gallon aquaria with two males of the desired strain for 20 days, at which time the females were isolated to tanks containing sufficient *Nitella* or *Ceraptopteris* to protect the young from cannibalism. Upon the birth of a brood, the female was removed and remated with the desired male after a 24-hour rest period. The progeny of each mating were counted shortly after birth. Embryos that never became fully activated were probably eaten by the female parent shortly after birth, but the evidence is good that most viable young escaped cannibalism.

Results and Discussion

In order to test the viability or vigor of mutant sperm in the presence of ovarian tissue, mutant female fish were initially mated with two mutant males. The young of the first brood were all mutant as was expected. Upon refertilization with two normal males, the second brood consisted of both mutant and wild-

*Present address: Department of Biochemistry, Tulane University, New Orleans, La.

type young. The ratios of mutant:wild-type young are presented in Table I, demonstrating the superior fertilizing power of wild-type sperm. It was found that 83 percent of the young of the second brood were heterozygous individuals, while only 17 percent of the progeny were mutants resulting from fertilization by retained sperm from the initial mating.

The ability of mutant sperm to replace wild-type sperm was demonstrated by a reversal of the breeding procedure so that a mutant female was initially mated with two wild-type males. After the birth of the first brood (all wild-type heterozygous progeny) the reimpregnation was accomplished with mutant males. In contrast to the first experiment, only 32 percent of the progeny were mutant (Table II) while 68 percent of the young were heterozygous individuals resulting from fertilization by retained sperm.

The average brood interval for *Lebistes* under the conditions of light and temperature existing in this laboratory is about 30 days. The freshest sperm retained in the ovary from the first fertilization was, therefore, at least ten days old before they were brought into competition with new sperm of a different genotype, assuming that the last copulation had occurred on the twentieth day. It is apparent that mutant sperm are less viable than wild-type sperm and that wild-type sperm retain their vigor to a greater extent than the mutant sperm when lodged in ovarian tissue.

Although the lordotic mutation does not appear to hinder the fish, the ability of mutant males to copulate in the presence of wild-type males was studied. To rule out the possibility of an occassionally infertile female fish, they were initially mated with two mutant males to ensure good fertility in the females used. The data presented in Table III demonstrate the results of an experiment in which a mutant and a wild-type male were used to fertilize a female previously mated with mutant males. Such a mating procedure resulted in a mixture of progeny in which 63 percent of the young were wild-type heterozygotes, in contrast to 83 percent replacement when both males were of the wild-type (Table I). This finding indicated that either fresh mutant sperm was deposited or that the quantity of wild-type sperm was low due to sexual interference by the mutant male. A third mating with two wild-type males after the second brood resulted in complete replacement of mutant sperm, again indicating the reduced viability of mutant spermatozoa retained in the ovary. A fourth brood, produced from mating the females with a mutant and a wild-type male, resulted in 16 percent replacement, thus demonstrating that successful copulation by mutant male fish occurred in the presence of wild-type males.

Although quantitative significance cannot be attached to the experimental results, it would appear that mutant male *Lebistes* are sexually active and are physically capable of copulation in a competitive environment. Since no marked differences in sexual activity, gross morphology or motility of the sperm cells could be found between mutant or wild-type fishes, the semi-lethal character can be assumed to result from some biochemical alteration during maturation of the spermatocytes.

Summary

1. The sperm of the lordotic *Lebistes* is less viable than the wild-type, indicating a semi-lethal condition associated with the mutation, *lordosis*. Replacement of mutant sperm is easily accomplished by reimpregnation of female fish with wild-type sperm but replacement of wild-type sperm by mutant sperm is not as easily accomplished.

2. The lordotic *Lebistes* male is capable of fertilizing female fish in the presence of wild-type males in spite of its anatomic discrepancy.

3. It is postulated that the decreased viability of mutant sperm is due to a biochemical alteration of the male sex cells, an alteration

TABLE I. The *in vivo* replacement of mutant sperm (S_cS_c) by wild-type sperm (S_nS_n) in mutant female guppies (S_cS_c).

Number and Strain of males	Brood	Ratio of mutant: wild-type progeny			Average percent Replacement
		♀75	♀76	♀79	
2 Mutant S_cS_c	1	5/0	4/0	7/0	
2 Wild S_nS_n	2	1/16	4/17	5/17	83

TABLE II. The *in vivo* replacement of wild-type sperm (S_nS_n) by mutant sperm (S_cS_c) in mutant female guppies (S_cS_c).

Number and Strain of males	Brood	Ratio of mutant: wild-type progeny				Average percent Replacement
		♀50	♀62	♀64	♀65	
2 Wild (S_nS_n)	1	0/6	0/7	0/10	0/15	...
2 Mutant (S_cS_c)	2	12/27	3/10	7/5	3/11	32

TABLE III. The *in vivo* replacement of mutant (S_cS_c) and wild-type sperm (S_nS_n) in mutant female guppies (S_cS_c).

Number and Strain of males	Brood	Ratio of mutant: wild-type progeny				Average percent Replacement
		♀21	♀24	♀25	♀26	
2 Mutant	1	21/0	16/0	16/0	12/0	
1 Mutant + 1 Wild-type	2	13/18	14/18	9/24	12/22	63
2 Wild-type	3	0/7	0/39	0/22	0/25	100
1 Wild-type + 1 Mutant	4	1/3	1/18	5/13	1/9	16

that is not morphologically apparent.

Literature Cited

1. Clark, E., L. R. Aronson and M. Gordon. *Anat. Rec.* (Abstract 89) 101:692. 1948.

2. Haskins, C. P. and E. F. Haskins. *Heredity.* 2:251-262. 1948.

3. Rosenthal, H. L. and R. S. Rosenthal. *Jour. Hered.* 41:217-218. 1950.

4. Van Oordt, G. J. *Regan. Tijd. Ned. Dierk-Vereen.* 1-2:77-80, 1928.

(*Received June 4, 1951*)

◆

A SURGICAL TECHNIQUE FOR THE RECIPROCAL TRANSPLANTATION OF FERTILIZED EGGS

In the Rabbit

Frederick R. Avis and Paul B. Sawin*

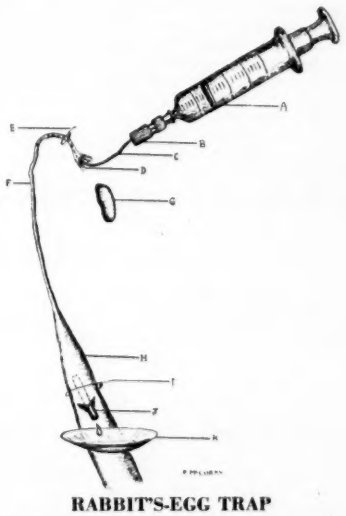
Several investigators, (Pincus[2], Chang[1], MacNutt (unpuolished) and others) have successfully reciprocally transplanted fertilized eggs in the rabbit but in such cases the donor was sacrificed to obtain the eggs. The technique described herein was developed because the study of the effects of uterine environment necessitates close control of the genetic constitution of the fertilized ova to be transplanted. The maximum uniformity can be secured only by using inbred races and by obtaining as large numbers of fertilized ova as possible from the same does. The latter necessitates the use and reuse of the same donor and recipient for several transplants. The present records show that 81.3 percent of the transplanted eggs have developed into normal offspring by this technique.

Preparation of Animal

The donor is mated normally with the selected buck. The recipient at this time is given 50 R.U. of gonadotrophin from the anterior lobe of sheep pituitary intravenously to produce ovulation. The does are ready for surgery twenty-four hours later.

Surgical Procedure

Careful and complete aseptic technique is used throughout the operation. The donor is anaesthetized with ether and bilateral incisions approximately two inches in length made ventrally over the sites of the ovaries and tubes. The latter are delivered by thumb forceps applied to the fat pads to which the organs are



RABBIT'S-EGG TRAP

Figure 19

A—Syringe; *B*—boist adapter; *C*—ureteral catheter; *D*—fimbriae; *E*—ligature; *F*—right fallopian tube; *H*—right horn of uterus; *I*—ligature; *J*—gum rubber tubing; *K*—watch glass. Over 80 per cent of the eggs captured by this apparatus are successfully implanted in "foster mothers" and come to normal birth.

*Head of Biology Department, Saint Mark's School, Southborough, Mass. and summer investigator, Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine; and Research Associate, Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine, respectively.

The authors wish to express their appreciation to Dr. Clarence C. Little for his helpful suggestions, to Dr. Meredith Runner for his help in constructing the glass pipettes used in this technique, to Mrs. Avis and Mr. Avis's many students who have helped as assistants and anaesthetists in these operations, and to Philip E. McCurdy, a member of the Secondary School Summer Program of the Jackson Memorial Laboratory for his illustration of the technique (Figure 19).

attached.

The procedure then follows the method illustrated in Figure 19. A V-shaped incision is made on the side of the horn of the uterus approximately one inch below the junction of the horn and fallopian tube. A short piece of gum rubber tubing is then inserted into the lumen of the horn and ligated in place. A strip of ureteral catheter approximately 1 mm. in diameter is slipped through the ostium and held in place by a ligature. Isotonic salt solution with 5 percent dextrose at 37°C. is then flushed slowly through the tube and some twenty drops collected in a watch glass held at the tip of the gum rubber tubing. The ligatures, catheter and tubing are then removed. In the authors' experience it has been found better not to put a suture on the v-shaped incision of the horn. The flap heals nicely by itself without adhesions. The peritoneum and muscle layers of the donor are then closed with a continuous suture (#000 gut) and the skin is closed with interrupted sutures (#00 black silk). The wounds are liberally swabbed with 5 percent tincture of iodine.

The recipient is anaesthetized with ether and bilateral incisions similar to those previously described made in her. The ovaries are inspected for ruptured follicles which must be present for successful transplant. It is advisable to have more than one recipient prepared in the event the selected doe has not responded to the gonadotrophin. If ruptured follicles are present, the donor's eggs are then drawn up in a fine glass pipette. Less than 0.5cc of solution should be used, and it must be free of bubbles. The pipette is slipped through the ostium and held in place by fine, curved, blunt thumb forceps. The contents are then gently blown into the fallopian tube, care being taken that no bubbles enter the tube. The incisions are closed and treated in the same manner as those of the donor.

Literature Cited

1. Chang, M. C. *Jour. Exper. Zool.* Vol. 114:1.
Vol. 77, No. 1. 1940.
2. Pincus, Gregory. *Anat. Record.* 77:1. 1940.

(*Received August 9, 1951*)

◆

NINTH INTERNATIONAL CONGRESS OF GENETICS

Scheduled to Take Place in Italy, August 1953

THE address of the Secretary of the 9th International Congress of Genetics, which will take place at the end of August, 1953, in Bellagio (Como), Italy, is: ISTITUTO DI GENETICA, UNIVERSIA DI MILANO, Via Celoria 10, Milan, Italy.

◆

THREE IN ONE: PHYSICAL ANTHROPOLOGY, GENETICS, STATISTICS

(*Continued from page* 256)

Study of Man, The Concept of Race, Use of Blood Groups in Human Classification, Man's Past, Man's Future. Scattered among these aggressive and subjectively colored chapters are others which report the findings of genetics in an unobjectionable manner. Here Prof. Boyd is not concerned with the "revolution" which he sees in physical anthropology; he has no novel idea to sell to an unbelieving public. Contrast, then, the titles of these chapters with those given above: Genetics, Heredity and Environment, Gene Equilibrium without Evolution, Factors Modifying Gene Frequencies (Evolution), The Influence of Geography on Racial Distribution, Blood Groups, Other Human Genes, Incompletely Analyzed Genetic Characteristics.

Prof. Boyd writes well and is able to present the intricacies of genetical theory in a clear fashion. There is real need for such a book, especially for use in colleges. It is to be hoped, therefore, that a revision will be undertaken at an early date, with a single coherent book emerging.

T. D. Stewart
U. S. National Museum

RACIAL DIFFERENCES IN PALM-PRINT RIDGE COUNTS

The *a-b* Ridge Count in the Ontario-British, European Jews and Ontario-Indians

T. C. FANG*
Department of Zoology, University of Toronto

THE fine lines that corrugate the fingers and palms of our hands and the toes and soles of our feet are called epidermal (or dermal or skin) ridges. The morphology of epidermal ridges is characterized by the presence of triradii. A typical triradius is the meeting point of three ridges called radiants making approximately 120° with each other. The three radiants do not, however, often meet. When they do the meeting point is known as the triradial point.

Generally, there are four digital triradii on a palm. These are situated just below the four fingers and are called *a, b, c* and *d*, beginning with the one under the index finger (Figure 20). The *a-b* ridge count, called the *a-b* count for short, is the number of epidermal ridges crossing or touching a fine straight line drawn joining the digital triradii *a* and *b*, nascent ridges being excluded. Following the conventions of ridge counting established by Bonnevie[1] and by Cummins and Midlo,[2] the triradial point or the radiant (when the former is absent) is not included in the count.

The *a-b* count of a person is the total of the *a-b* counts of the two hands. This ranges from below 70 to over 100. Counts of 78 or below are classified as "low"; those of over 78 are "high."

The genetic factors which determine variation in the *a-b* count have been analyzed and consist of a main gene, whose expression is affected by multiple modifying factors. The allele which determines a high count (79 ridges and over) is dominant over its allele determining a low count. Modifying factors affect the place in the high or low category the individual occupies.

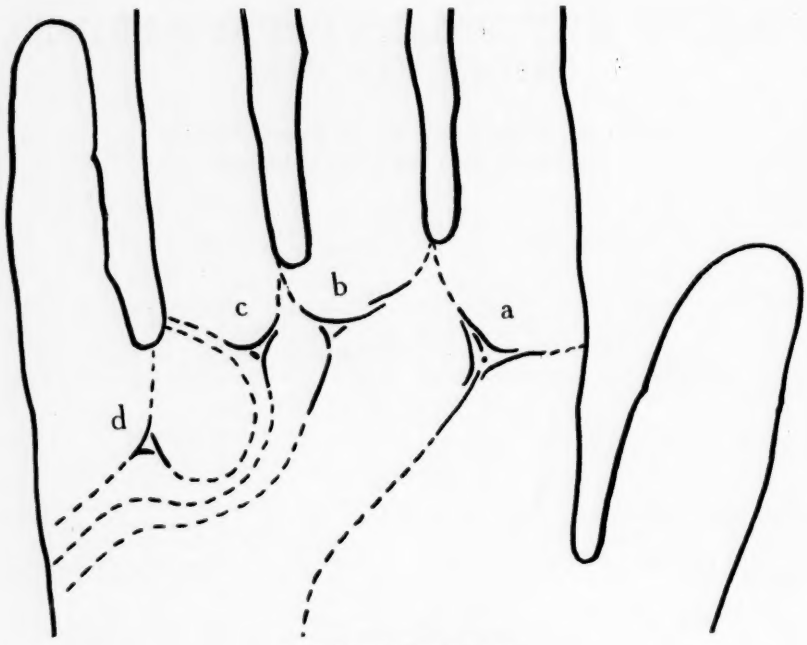
Source of Cases

The present note records, for the first time, the study of this character in three Canadian populations having different ethnic origins: Ontario-British, European-Jewish and Ontario-Indian. The Ontario-British population consisted of white residents of Ontario having English, Scottish or Irish names. A few of them may not be British by descent as people other than British may occasionally have an English or Scottish name. The palm prints of this sample were collected during two exhibitions held in the years 1940 and 1947 in the Department of Zoology, University of Toronto.

The group classified as European Jews consisted of Jews who came from Europe themselves, or whose parents did. According to the place of birth of their parents, they were roughly divided into three groups: A) Russian, B) Polish and C) others—whose origins were in other parts of Europe, e.g. Germany, Austria, France and England. This last group is very heterogeneous with respect to place of birth. They were classified together simply because the number of cases is very small.

The Ontario-Indians studied consist of two groups. The one called "Indians P.S." (Pekangecum Saulteaux) was derived from Indians living in the Berens River district, near the boundary of Ontario and Manitoba. The other, called "Indians M.I.," consisted of school children in the Mohawk Institute, Brantford, Ontario. The former is thought to possess "pure blood" for they are living in an area not easily accessible and no known intermarriage with Whites or with other racial groups has occurred. The latter group is known to have some degree of "mixed blood" as a result of intermarriage of some of their

*Holder of Canadian Red Cross Scholarship. The writer is very grateful to Dr. Norma Ford Walker for suggestions and criticism. Thanks are also due to Miss Muriel Smart for her valuable help.



LOCATION OF TRIRADII

Figure 20

Semi-diagrammatic tracing of palmar print, showing location and variations in form of the five digital triradii (*a-d*). The present study analyzes the distribution of the number of ridges between the triradii *a* and *b*.

ancestors with Whites.

The palm prints of these populations can be regarded as random samples in the sense that they were collected without any preference or bias on the part of the collectors and no known relatives were included.

The number of prints used in this study totals 445, distributed as follows: Ontario-British, 160; Russian Jews, 64; Polish Jews, 68; other European Jews, 41; Indians M.I., 72; and Indians P.S., 40. The proportion of sexes is shown in Table. I.

All the materials were collected by Dr. Norma Ford Walker of the University of Toronto and her associates, and are part of the permanent university collection.

Observation

The results of this investigation are summarized in Table I. Some results of an earlier study made in England by the writer on the *a-b* count in the general British population are here included for comparison.

1. The mean count is significantly higher in the Ontario-British (85.88 ridges) than in the British population sampled earlier (83.03 ridges). The mean difference is 2.85 with a standard error of 0.88.

2. The mean count of the European Jews, 80.24 ridges, is low. So is that of the Indians M.I., 80.51 ridges. In either case it is significantly lower than that of the British, Ontario-British or Indians P.S.

3. In terms of the low count class frequency, this is lowest in the Ontario-British and highest in the European Jews.

4. No significant sex differences in the mean counts in all the samples studied have been found.

Discussion

The difference between the two Indian groups may be due to some racial intermarriage, which occurred in one of them, and to other factors, while the idea of racial variations may be used to account for the similarity in the Jewish groups and the differences among the Ontario-British, British, Jews and Ontario-Indians. But how is the difference between the Ontario-British and British in England to

be explained if the Ontario-British are mainly British living in Ontario?

Several explanations are possible:

1. The Ontario-British sample might have contained many persons who were not British by descent. If this were the case, what the effect of the mean ridge count may have been is not known. This contingency does not seem very likely. People of British origin are in a great majority in Ontario, and especially in Toronto.[3] Since the sample was selected to include British, it seems unlikely that it contains many non-British.

2. The Ontario-British sample contained many persons with Scottish names, while the earlier sample of the British population was collected in London. Should Scottish people have a higher mean count than the English (which is possible) this may be responsible for the difference in the mean counts observed in the British and Ontario-British samples.

3. If there is no significant difference in the *a-b* count between the English and the Scottish populations, and if the collection is not a random sample for the general Ontario-British population, the difference observed may be simply one among Ontario-British themselves and not between the Ontario-British and the British in England. In view of the fact that the prints were collected from those persons who came to see the exhibitions mentioned before, they may not constitute a true random sample. People who come to see an exhibition in a university may have some inclination related to academic activities more pronounced in them than in the population as a whole. If this kind of mental trait should somehow or other be related to the *a-b*-count, the sample might deviate considerably from the mean. In that case it would not be representative of the *a-b* count of the entire Ontario-British population.

4. If the selected collection should actually be a random sample of the Ontario-British, and if there is no significant difference in the mean *a-b* count of the English and the Scottish, then the present data indicate that more British with a high *a-b* count than with a low *a-b* count have immigrated to Ontario. This might be because some mental trait accentuated in the immigrants is correlated wth the *a-b* count. It is thus seen that the result of either supposition, whether the collection is a random sample for the Ontario-British or not, tends to suggest the same thing: there is some relation between the *a-b* count and certain mental characters.

It is interesting to note that the author found the mean *a-b* count of 204 English students in the Universtiy of London to be 84.3 with a standard deviation of 10.4. This agrees well with that of the Ontario-British. The agreement between these two samples might be due merely to chance. But the coincidence does lend color to the above assumption.

TABLE I. Mean *a-b* ridge counts with standard deviations and percentage frequencies of the low count class in the British, Ontario-British, European Jews and Ontario-Indians.

Sample	Sex	No.	Mean	S.d.	% of "low" (78 or less)
British	F.	435	83.01	9.7	26.7
	M.	424	83.04	10.3	29.7
	Av.	859	83.03	10.1	28.3
Ontario-British	F.	83	85.54	10.2	24.1
	M.	77	86.25	10.5	27.3
	Av.	160	85.88	10.3	25.6
European Jews:					
Russians	F.	38	80.16	9.7	42.1
	M.	26	81.38	11.2	42.3
	Av.	64	80.66	10.3	42.2
Polish	F.	27	79.98	7.7	40.7
	M.	41	79.74	13.0	51.2
	Av.	68	79.84	11.1	47.1
Other	F.	18	81.56	13.2	36.7
	M.	23	79.28	11.4	52.2
	Av.	41	80.28	12.0	42.4
TOTAL	F.	83	80.41	9.9	39.8
	M.	90	80.10	12.2	48.9
	Av.	173	80.24	11.1	44.5
Indians M.I.	F.	43	81.08	10.5	32.6
	M.	29	80.00	9.1	37.9
	Av.	72	80.51	9.9	37.4
Indians P.S.	F.	14	85.64	9.9	14.3
	M.	26	83.42	8.7	34.6
	Av.	40	84.20	9.0	27.5

Literature Cited

1. Bonnevie, K. *Jour. Genet.* 15:1. 1924.

2. Cummins, H., and C. Midlo. Finger Prints, Palms and Soles. Blakiston Co., Philadelphia. 1943.

3. Dominion Bureau of Statistics. 1945. Racial Origin. *Canada Year Book,* 1945 , pp. 103-107.

4. Fang, T. C. *Jour. Ment. Sci.,* 95:945. 1949.

5. ————. The inheritance of the *a-b* ridge count on the human palm. (A Ph.D. thesis, University of London.) 1950.

(Received July 12, 1951)

HYBRIDIZATION IN THE TAPEWORM

"Out-of-step" embryos of the Cestode *Hymenolepis pitymi* Yarinsky due to crossing between strains or species*

ARTHUR W. JONES

The flat worms impinge variously on the affairs of the human species. Because some cestodes are dedicated to realizing a more abundant life in the human intestine, they are a public health problem of considerable importance in some countries. Other flat worms infect livestock and domestic animals with untoward results to the farmer and the breeder. To the experimental biologist the cestodes present some challenging enigmas. The part that copulation and fertilization plays in the growth of populations of these worms is still largely unknown.—ED.

THE linearly budding cestode—in the host's intestine—is in one sense, a "population" of separately reproductive segments—the strobila. The genetic and evolutionary effects of self- or intercopulation of strobilae are important. Each segment of a cestode is equipped at different stages of the life cycle with both male and female sex organs. The manner in which these organs function has important evolutionary implications. If the cestode "population" must remain completely isolated, reproducing only by self-fertilization, homozygosity must be usual; and hybridization as a factor in speciation must be ruled out. But if cross-fertilization can occur, then speciation in cestodes (a rather rapid speciation, according to Baer[1]) may be partly explicable in terms of recombination.

Most workers believe, as does Hyman[2], that each segment of a cestode ordinarily is self-fertile. Yet the possibility of copulation between different segments, as well as between different worms, is recognized. The fact that in many cestodes the testes and other male organs mature before the parts of the female system in the same segment suggests that self-fertilization must be at least delayed in these species, and that cross-fertilization may to that extent be favored; but the usual appearance of the complete strobila of such cestodes is one of even and gradual development, there being only imperceptible changes as the maturing segments grow away from the scolex, the eggs of each succeeding older segment being slightly more mature than those of the next younger segment.

In the few cestodes where the female systems mature sooner than the male, and where, *a priori*, segments which cross-fertilize would reproduce earlier, there seems to be no specific evidence of cross-fertilization. In the monoecious members of the Acoleidae, however, where no female pore exists, hypodermic impregnation, more or less at random, may result in occasional cross-fertilization. And according to Smyth[4] specimens of *Schistocephalus* sp. *in vitro* expel spermatozoa into the medium, without penetration of vagina by cirrus, yet with production of some fertile eggs (in, presumably, any segments containing maturing ova). Whether such copulatory behavior occurs when *Schistocephalus* occupies a host in the normal manner is unknown.

Observations on *Hymenolepis Pitymi*

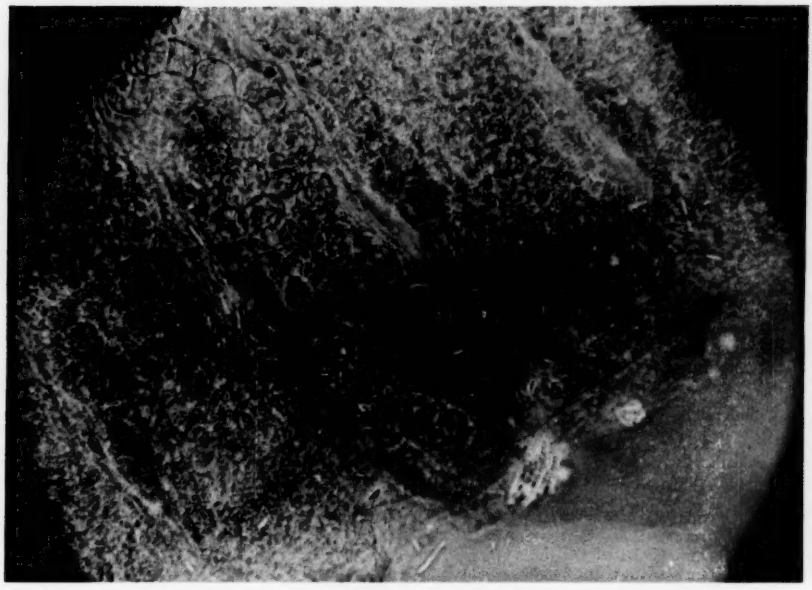
Seventy-five cestodes, most of them belonging to *Hymenolepis pitymi*, recently described by Yarinsky[5], were recovered from the intestine of a pine mouse trapped in Knox County, Tennessee. An examination of whole mounts of complete specimens revealed that a few of the worms have some segments, in the region of developing embryos, whose embryos are 'out-of-step"—either more developed or less developed than the embryos of neighboring segments. Of the twenty-nine specimens examined, five contain segments with embryos older than those in adjacent segments (Figure 21.), while one contains a segment with embryos younger than those adjacent. The occurrence of such "out-of-step" segments requires some explanation, since these cestodes, like most Taenioids, otherwise exhibit the expected gradualness of development.

Interpretation

Two explanations may be suggested.

First the actual age of the embryos in question may be different from that of the adjacent

* Contribution number 52, the Department of Zoology and Entomology, The University of Tennessee.



MIS-TIMED SEGMENTS

Figure 21

Photograph of gravid segments of *Hymenolepis pitymi*, showing "older" embryos between two groups of "younger" embryos. Since normal development is rigidly timed to the definite age-sequence of the segments, it is suggested that these aberrant developmental rates may be due to hybridity.

embryos. That is, their beginning as zygotes may have been, in five cases, earlier and in one case later, than the beginning of neighboring zygotes. This suggestion implies some interference with the copulation and/or fertilization-mechanism, speeding it up in segments of five worms, and slowing it down in a segment of one worm. But no known factors can account for such hypothetical action. The cestode strobila is both genetically and environmentally uniform. It is the product of vegetative growth, carried on through mitosis (see Jones[3]); all parts of it except the gametes and gametocytes in meiosis should be genetically the same. The host intestine is a restricted habitat in which all segments of the worm share a uniform environment. Therefore it is hard to conceive of genetic or environmental conditions which might alter any segment without affecting the adjacent regions. Accordingly the actual age of the embryos in question must not be different from that of the neighboring embryos.

An alternate explanation remains, which considers that the embryos themselves, not their age or their environment, are intrinsically different from their neighbors. This difference is expressed in their rate of growth, and is due to their being the results of hybridization.

The host in which these cestodes lived was a small mammal, in whose intestine the worms were crowded, nearly occluding the lumen. This crowding would give opportunity for cross-copulation. Moreover, there was at least one other species of *Hymenolepis* beside *H. pitymi* present. It is thus conceivable that crossing took place. Such mating would produce "hybrid" zygotes in the segments inseminated, whether the cross-breeding involved members of different species, or only members of different strains of the same species. Such zygotes would be genetically different from their neighbors. They would also be uniform in any given segment, as is expected in an F_1 generation, and as in fact occurs in these worms. The F_1 might express its uniqueness in several ways, among which would be difference in growth rate. (In the observed cases, only one group of embryos failed to show "heterosis," regarding rate of growth.) This difference, moreover, as Figure 21 illustrates, is obvious. The hypothesis of cross-fertiliza-

tion, or hybridization, seems the only tenable explanation.

It is probable, therefore, that cross-fertilization does occur in cestodes. The isolation of cestode populations may thus be capable of interruption; and recombination through hybridization may be a factor in cestode speciation.

Summary

The occasional occurrence of groups of advanced or retarded embryos in cestodes is explainable as the result of crossing between members of different strains or species, in the absence of other reasonable hypotheses.

Literature Cited

1. Baer, J. G. Ecology of Animal Parasites. Univ. of Illinois Press, Urbana. 1951.
2. Hyman, L. H. The Invertebrates: Platyhelminthes and Rhynchocoela. Vol. I. McGraw-Hill, N. Y. 1951.
3. Jones, A. W. *Jour. Parasit.* 31: 213-235. 1945.
4. Smyth, J. D. *Jour. Parasit.* 36: 371-383. 1950.
5. Yarinsky, Allen. *Jour. Tenn. Acad. Sci.* (in press.) 1951.

(*Received July 25, 1951*)

◇

PROGENY TESTS AND WARWICK'S TABLES

THE note by Kidwell[1] on the numbers of progeny necessary to test a sire for heterozygosity for a recessive autosomal gene is both useful and timely. From what Mead *et al*[2] found in a random sample of only six bulls, it is certain that some of the bulls now being used so extensively for artificial insemination are transmitting some bad genes as well as good ones. Eventually cattle breeders may have to be more concerned than they now seem to be with the testing sires for genes that are lethal or otherwise undesirable.

Kidwell's note gives the minimum numbers necessary to establish a probability of only five percent that the animal tested is not heterozygous for a recessive gene after various matings in which a heterozygote should yield ratios of 1:1, 3:1 and 7:1. While these numbers are probably as good as one may hope for in the larger domestic animals, there are some species in which tests can be made at the one percent level of significance with so little extra trouble and expense that most geneticists would hardly be content to stop at the five percent figure.

The domestic fowl is one of these. One needs only five chicks with rapid feathering to yield a probability of $<.05$ that their sire does not carry the gene *K* for slow feathering, but with two more that probability becomes $<.01$. Most of us would hatch at least seven.

There are also cases in which one will wish to know the chance that some sire or dam is or is not a carrier when the number of offspring on which that chance may be estimated is either more or less than the exact number that yields a probability of $<.05$. When a litter of puppies contains only three males, all normal, the probability that their mother does not carry haemophilia is .125, but if the litter contains 6 normal males that probability becomes only .0156.

The purpose of this note is to call attention to a helpful first-aid that provides probabilities for all contingencies. The extensive tables compiled by Warwick[3] give for nine different Mendelian ratios the probability of getting by chance any ratio observed in test populations numbering from 1 to 50. For any of these 9 ratios one may see at a glance the numbers needed to yield probabilities of $<.05$ and $<.01$. With equal facility one may determine whether any observed segregation fits better an expected ratio of 3:1 or of 2:1, of 1:1 or of 9:7.

Having gratefully used these tables for 19 years, the writer is prompted by the recent note in the Journal to bring them to the attention of geneticists to whom they may have been hitherto unknown. While so doing, he wishes to thank Dr. Warwick for the help given for all these years, and to express the hope that the Texas Agricultural Experiment Station will continue to supply (without cost) his useful publication.

F. B. Hutt

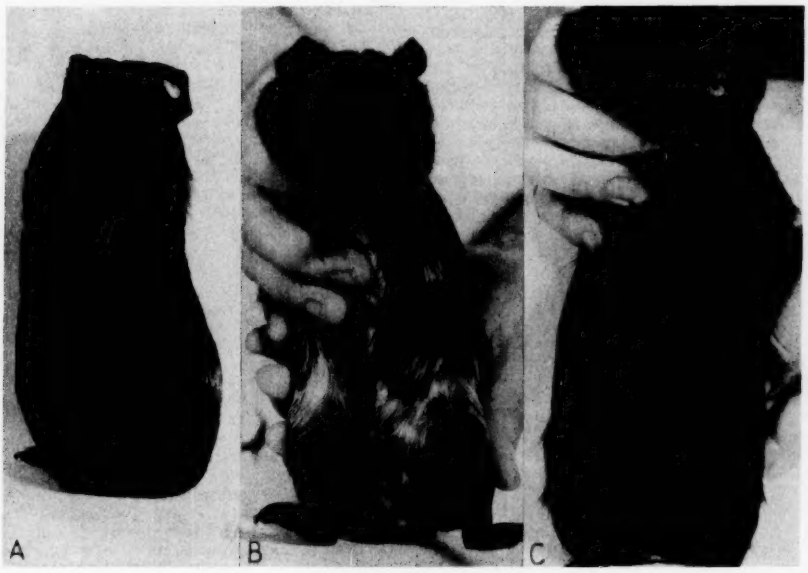
Cornell University
Ithaca, New York

Literature Cited

1. Kidwell, J. F. *Jour. Hered.* 42: 215-216, 1951.
2. Mead, S. W., P. W. Gregory and W. M. Regan. *Genetics* 31:574-588. 1946.
3. Warwick, B. L. Probability Tables for Mendelian Ratios with Small Numbers. *Texas Agric. Exper. Sta. Bull.* 463. 28 pp. 1932.

ROAN, A MODIFIER OF PIGMENTED HAIRS IN GUINEA PIGS

HEMAN L. IBSEN AND BETTY LANNING GOERTZEN*



ROAN GUINEA PIGS

Figure 22

A—Dorsal view of a black individual showing extreme roaning (*Ro Ro*). *B*—Ventral view of the same animal showing completely white areas in the inguinal region. *C*—A black heterozygous roan (*Ro ro*) showing only a few roan hairs ventrally.

THE roan gene (*Ro*), producing an intermixture of white with pigmented hairs in blacks, reds, and chocolates, was described by Ibsen[1] in a report listing some of the genetic factors in guinea pigs. It was assumed to be incompletely dominant to its absence. A similar condition, silvering, was reported by Wright[2] and was attributed, in the main, to an incompletely recessive gene (*si*).

Description

Roan is almost always present at birth and usually persists unchanged with age. The degree of expression varies from animals having a predominantly white appearance, with some areas in the inguinal region that are completely white (Figure *22B*), to those having only a very few white hairs (Figure *22C*).

Method

In order to study the inheritance of roaning, matings were made involving extremely roan, slightly roan, and non-roan animals. The data

* Contribution No. 165, Department of Animal Hubsandry, Kansas State Agricultural Experiment Station, Manhattan, Kansas.

were accumulated only from matings between blacks since it was much easier to distinguish the presence of white hairs in slightly roan blacks than in slightly roan chocolates or reds. Even with this precaution the presence of a few white hairs was either overlooked in a small number of animals or showed a delay in expression, and was found only after the animals were rechecked at a later date.

Hair samples were taken from the back, sides, and belly of the animals and placed in an envelope. A miniature diagram of the animal on each envelope was used to indicate the locations from which the samples were taken. Each envelope also contained the pedigree number of the animal, the date at which the sample was taken, and a brief description of the animal. The first hair samples were usually taken when the animal was about two months old. In a number of cases other hair samples were taken as the animal grew older in order that the persistence of the character could be checked.

Inheritance

An incompletely dominant gene (*Ro*), with a minus modifier or modifiers producing a variation in the degree of roaning, explains the inheritance of roaning in our stock.

Only roan progeny were produced when extreme roans (*Ro Ro*) were mated to slightly roan animals (*Ro ro*) with nine showing slight roaning (*Ro ro*) and seven extreme roaning (*Ro Ro*) (Table I, row 1).

When very roan animals (*Ro Ro*) were mated to non-roans (*ro ro*), 13 slightly roan animals (*Ro ro*) and one non-roan were produced (Table I, row 2). A minus modifier or modifiers could explain the one non-roan offspring, a male. Unfortunately the latter was not tested genetically.

Matings between slightly roan animals (*Ro ro*) produced 129 roans and 46 non-roans. The observed ratio comes unusually close to the expected 3:1 ratio, 131.25:43.75 (Table I, row 3). Assuming incomplete dominance, one would have expected that one-third, or about 43 of the above 129 roans, would be *Ro Ro*. Actually only 16 were classified as such. Our explanation of this discrepancy cannot be proved, but it is our assumption that a minus modifier caused the reduction in the number of white hairs. This made some *Ro Ro* animals look like *Ro ro*, and shifted a few *Ro ro* animals into the *ro ro* category. In one mating (Table II, row 18) both heterozygous parents were at first classified as non-roans (*ro ro*). Later roan hairs were observed near the mammae, and the classification was changed from *ro ro* to *Ro ro*. Presumably both of these parents had minus modifiers which greatly reduced the amount of roan. Twenty-three of their 30 offspring did not show roaning.

Some of these so-called non-roans would be expected to be heterozygous roans (*Ro ro*) with minus modifiers completely inhibiting the expression of the roan gene. Some of the animals classified as heterozygous (*Ro ro*) would also be expected to be homozygous roans (*Ro Ro*) with the minus modifiers again caus-

Table I. Progeny from five monohybrid matings involving homozygous roans (Ro Ro), heterozygous roans (Ro ro), and non-roans (ro ro).

ROW	PARENTS	OFFSPRING	Ro Ro	Ro ro	ro ro	TOTAL
1	Ro Ro x Ro ro	Observed No.	7	9		16
		Expected No.	8	8		
2	Ro Ro x ro ro	Observed No.		13	1	14
		Expected No.		14		
3	Ro ro x Ro ro	Observed No.	16	113	46	175
		Expected No.	43.75	87.50	43.75	
4	Ro ro x ro ro	Observed No.	1	84	90	175
		Expected No.		87.50	87.50	
5	ro ro x ro ro	Observed No.		14	107	121
		Expected No.			121	

Table II Progeny from 21 matings of heterozygous roans (Ro ro). Shows variation in ratios for individual matings.

ROW NO.	OFFSPRING Ro Ro	Ro ro	ro ro
1	2	3	
2	5	9	1
3		8	2
4		3	1
5	1	1	1
6			1
7		1	2
8	1	2	
9	1	6	
10		4	
11	1	1	
12	1	1	1
13	1	6	2
14		21	1
15	2		
16		9	2
17		13	1
18		7	23
19	1	11	4
20		6	2
21		1	2
TOTAL	16	113	46

Table III Showing the 14 heterozygous roan progeny (Ro ro) produced from matings between animals classified as non-roans (ro ro).

PARENTS "ro ro" x "ro ro"	OFFSPRING ro ro	Ro ro
♂P14.2 x ♀N381.1	17	1
♂P14.2 x ♀N381.1* ♂ ♀P701.1*	3	1
♂P14.2 x ♀ 5943	2	1
♂P567.2 x ♀P567.1	31	7
♂P567.2 x ♀P777.3	8	3
♂T367.2 x ♀V13.1	6	1
TOTAL	67	14

*The two females produced a "double-litter."

ing the animal to be classified erroneously. Progeny from another mating (Table II, row 14) also show a great deviation from the expected 1:2:1 ratio. Only one of the 22 progeny produced was classified as a non-roan (*ro ro*) and 21 as heterozygous roans (*Ro ro*). It is possible here, again, that one of the parents was a homozygous roan (*Ro Ro*) with minus modifiers reducing the amount of roan expressed.

In matings between non-roans (*ro ro*) and slightly roan animals (*Ro ro*) (Table I, row 4) 175 progeny were produced: 90 non-roans (*ro ro*), 84 heterozygous (*Ro ro*), and one apparently a homozygote (*Ro Ro*). The *ro ro* parent of the animal showing extreme roaning conceivably was given the wrong classification due to the presence of a minus modifier or modifiers. When the very roan animal is excluded, the data fit the expected 1:1 ratio; although there is again a slight excess of non-roans.

When non-roans (*ro ro*) from the roan stock were mated together, 121 progeny were produced, 107 showing no roaning (*ro ro*) and 14 some roaning (*Ro ro*) (Table I, row 5). The 14 heterozygotes came from several matings involving three sires and five dams (Table III). It is possible that one of the parents in each of these matings was a heterozygous roan that was phenotypical *ro ro* due to the minus modifier.

Summary

Roan, *Ro*, an incompletely dominant gene, causes an intermixture of white with pigmented hairs in black, red, and chocolate guinea pigs. The presence of a minus modifier or modifiers apparently reduces the expression in both homozygotes and heterozygotes, making the homozygous roans appear as heterozygotes, and in a few cases causing the complete absence of roan in heterozygotes. Roan is usually present at birth and persists comparatively unchanged with age. The degree of expression varies considerably.

Literature Cited

1. Ibsen, H. L. *Proc. 6th Int. Congress. Genetics* 2:97-101. 1932.
2. Wright, S. *Genetics* 32:115-141. 1947.

(*Received June 20, 1949*)

◆

THE EFFECT OF ALTITUDE ON GERM CELLS

In Relation to Cytological Technique

FOR many years cytologists have disagreed on the value of the various techniques used for the study of chromosomes in germ cells. Bouin's solution has proven its value for the chromosomes of insects. Allen's modification of Bouin's solution has been used for the spermatogonia and spermatocytes of vertebrates, but Matthey[3] gives it a poor or mediocre rank except for the study of amphibian chromosomes. Painter[5] has long advocated the use of Allen's modification of Bouin's solution for mammalian cytology. While working with Painter and later in independent work, this author [1, 2] found Allen's modification of Bouin's fluid to be an excellent fixative, especially for spermatogonial chromosomes of mammals, and for mammalian spermatogenesis in general. The Japanese[4] authors seem to prefer Champy's fluid, or some other fixation containing osmic acid, when working with reptilian and mammalian chromosomes.

In September of 1948 this author became associated with Texas Technological College at Lubbock, Texas, and shortly afterwards began work with local mammals, using testicular tissue for the study of chromosomes. The work was extended to the use of reptilian and avian tissue. The technique used was Allen's modification of Bouin's solution. Matthey has rated the method as mediocre for reptiles and mammals, and poor for birds. At Lubbock the method proved to be of no value whatever for chromosomal studies of either of the three groups.

HIGH ALTITUDE TETRADS
Figure 23

Photomicrograph of tetrads of a grasshopper prepared with modified Bouin's solution.

After trying the Bouin-Allen method on about 20 different animals, including five kinds of lizards and five kinds of mammals, the technique was carefully checked step by step to see if an error had crept in at any place. No error was found. The work had been done at various times from early spring until late summer, but not one good spermatagonium was encountered. In reviewing the work of

a class in laboratory technique it was found that slides made of grasshopper tests with the unmodified Bouin's solution were below standard for prophase stages, but were usable for metaphase stages of spermatocytes and spermatogonia. It was quite evident that some other factor had expressed itself in vertebrates, but not so evident in grasshoppers except in early diakinesis.

After considerable experimentation it seems that the unexpected factor is the effect of the altitude. Painter's work was done at about 500 feet above sea level. Matthey did not say where his work was done, but he is located at Lausanne, Switzerland, which is about 1,200 feet above sea level. The altitude at Lubbock is a little above 3,200 feet. It is interesting to note that Painter had excellent results with the Bouin-Allen method while working with mammalian tissue; Matthey reports mediocre results with mammalian tissue, while at Lubbock the method is worthless for the tissue of all vertebrates studied so far.

It was plainly evident that a new approach to the problem of technique must be made, if good results were to be obtained. The evidence pointed to a cellular adjustment in response to the effect of a high altitude, though the possibility has not been excluded that other environmental factors may be involved. All spermatogonia observed in the metaphase were so contracted that the study of separate chromosomes was virtually hopeless. Some early prophase stages were fair. Later prophase and metaphase stages in mammalian spermatocytes were poor.

Experiments were begun in August of 1950 to see if changing the proportions of Bouin's fluid would have any appreciable effect. The first animal used was the horned lizard of the genus *Phrynosoma*. The first experiment was merely a matter of trial and error. The fixative used was

60 cc. saturated aqueous picric acid
25 cc. glacial acetic acid
25 cc. formaldehyde
1.5 grams chromic acid
3.0 grams urea

Although this first attempt was based on the contracting effect of picric acid and the counteracting effect of acetic acid, the amounts used were determined by guess work. Fortunately, it was a good guess, and excellent spermatagonia were obtained. This method did not work well for mammals, even when the fluid was heated to 37°C. The mammal used was a house mouse. Later the tissue from a young (half grown) cotton-tail rabbit (*Sylvilagus*) was preserved. This time the following modification was used:

60 cc. saturated aqueous picric acid
25 cc. glacial acetic acid
35 cc. formaldehyde
3.0 grams chromic acid
3.0 grams urea

(The picric acid, acetic acid, and formaldehyde were mixed together and heated to 35°C. before the chromic acid and urea were added.)

Excellent results were obtained for spermatogonia but the rabbit did not show very much activity in spermatogenesis, probably because of its age.

When the experimental work was extended to include grasshopper testes, it was found that the unmodified Bouin's solution gave good results in very late diakinesis and metaphase stages. (Figure 23). Spermatogonia were fair to good. However, it was found when Bouin's fluid was changed to

60 cc. saturated aqueous picric acid
25 cc. glacial acetic acid
25 cc. formaldehyde

early stages of tetrads were very good. When this method was used all four chromatids of a tetrad could be distinguished easily. Such tetrads were not found when the unmodified Bouin's solution was used, but the unmodified solution gave better spermatogonia. So far no good results have been obtained in this experimental work when avian tissue was used.

In all cases dehydration was with graded alcohols and clearing was with xylene. The tissues were embedded and sectioned in paraffin; staining was with Heidenhain's iron-haemotoxylin. This work is still in the experimental stage for different altitudes.

Summary

When Allen's modification of Bouin's fluid was used for preserving reptilian and mammalian tissue at an altitude of 3,200 feet, the tissue was of no value for the study of chromosomes.

The evidence obtained in this work seems to indicate that germ cells of reptiles and mammals make very definite changes in response to conditions at higher altitudes. The change in the testicular tissue of grasshoppers is not so pronounced.

Techniques that have given good results for 3,200 feet above sea-level are made available for the preservation of reptilian and mammalian testicular tissues.

Literature Cited

1. Cross, J. C. *Journ. of Morph.* 52:2. 1931.
2. ———. *Cytologia* 8:3-4. 1938.
3. Matthey, Robert. *Les Chromosomes des Vertebres.* 1949.
4. Minouchi, O. *Jap. Jour. Zool.* 1:1928.
5. Painter, T. S. *Anat. Rec.* 27:2. 1924.

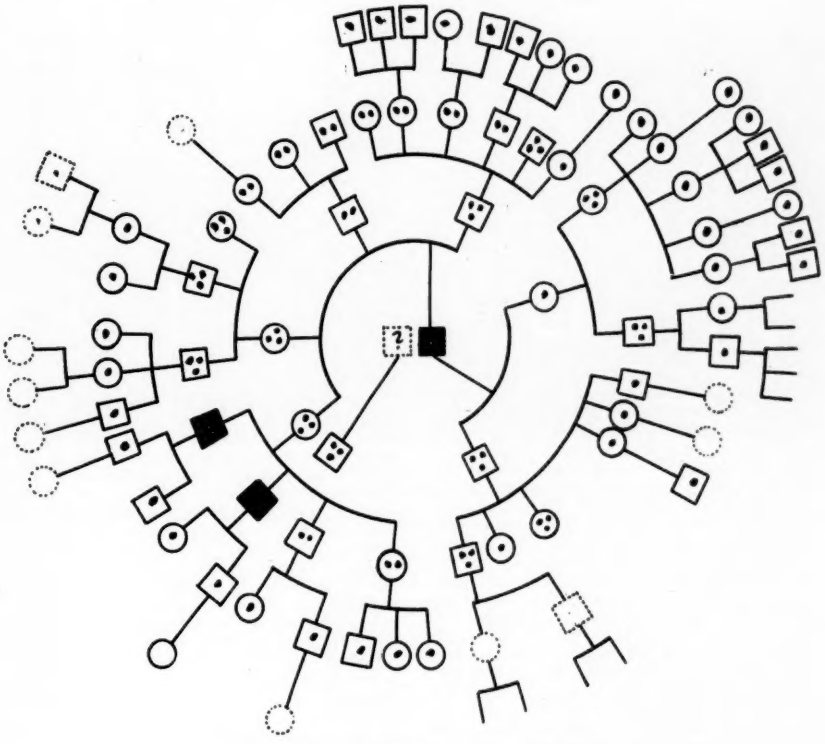
J. C. Cross

Texas Technological College
Lubbock, Texas.

(*Received May 14, 1951*)

A NOT DOMINANT GLAUCOMA PEDIGREE

RICHARD B. GOLDSCHMIDT
University of California, Berkeley



GENERATION-SKIPPING GLAUCOMA

Figure 24

Affected individuals are shown in black. One dot indicates that individual was under 50 when examined; two dots show individuals between 50 and 70; three dots show over 70. Many individuals in this pedigree are well over the age of incidence for glaucoma, which occurred in the early fifties in the individuals who were affected.

UP to very recently it has been assumed that glaucoma, both juvenile and senile, is a dominant trait. For a long time I have known a pedigree in which this is not the case, but have hesitated to comment upon it. Quite recently Waardenburg,[1] who had always assumed dominant heredity, published a number of pedigrees for recessive senile glaucoma. (The publication contains the clinical evaluation and a literature list.) Only a year ago I personally checked the pedigree to which I have referred and, since it rather remarkably

suggests sex-linkage, I propose to make it known. It deals with typical senile glaucoma, appearing in all stricken individuals in the early fifties; in one case it was diagnosed too late and led to blindness, in the second it had progressed far before it was discovered, but was kept in check, until the affected individual died from a kidney disease; in the third case it was found when just beginning and checked by medication.

The first recorded affected individual of this pedigree was blinded by glaucoma at the age of 57. He died soon after from an infection. His parents are known to have died as old people without eye trouble. His wife died at 82 with good eyes. The diagnosis of glaucoma had been made by nobody less than von Graefe, the classic scholar in this field. The patient had six children from two wives, three daughters and three sons. With the exception of one daughter who died relatively young (about 40) all lived to a mature age (65-83 years) without eye trouble. One daughter, who died at 81 with unusually good eyes, had three sons and one daughter. Two of the sons developed glaucoma between 53 and 55 years of age, like the maternal grandfather. In both cases, the diagnosis was made by internationally known European oculists (Igersheimer, and Steindorff). The older brother died of a kidney disease, half-blind from destruction of the greater part of the field. The younger one lives in advanced age with a stationary small dent in the inner field of one eye. Another brother died of a violent death at 61, one sister is alive and normal at 65. The two sisters of the mother of the two afflicted sons had three sons and two daughters, all normal at ages between 73 and 81. Thus out of all the grandchildren through daughters of the original case two males were affected and four not affected. The father of the affected brothers died at 69 with good eyes. His two brothers died at over 80 with good eyes and so did his parents; the father at about 70, the mother at 82. No other cases have appeared in the pedigree. At the last check there were 10 more unaffected members over 60 or even 70. The rest of the family is too young for a decision.

In the accompanying pedigree, the affected persons are shown in black. The normal ones are marked with 1 dot for persons below 50, two dots for 60-70, whether dead or alive, and three dots over 70. Young children are not marked. The pedigree is complete except for one child of the third generation who died in infancy from scarlatina.

Two explanations are possible. We have here either the first case of sex-linked glaucoma or a possibility of recessive inheritance. There is, of course, no reason why the first should not occur. As both glaucomatous brothers of the third generation do not have grandsons through daughters the case rests at this point. The second consideration requires that, by chance, the only marriage of a heterozygous child of the first case with a heterozygous partner was the one which produced the two instances of glaucoma. There was no consanguinity here nor anywhere else in the pedigree. But it is a fact that in the second generation the decisive marriage was the only one in which husband and wife came from old families of the same town; this certainly increases the chances for heterozygosity by remote common ancestors. If the inheritance were sex-linked the disease would have now disappeared completely from the family. If it is recessive, it might still reappear in the younger generations. As members of these generations have spread all over the world making possible marriages without even remote common ancestry, the probability for heterozygous matings has correspondingly decreased. There is no possibility to decide for one or the other interpretations on the basis of the small numbers. If a recessive is involved, the probability of a not-chance result is .2109, while in the case of sex-linked inheritance it is .2344. Thus a final decision will have to wait for the discovery of similar pedigrees.

Literature Cited

1. WAARDENBURG, P. F. *Genetica* 25:79-125. 1950.

(*Received May 14, 1951*)

Postdoctoral Fellowships Announced

THE National Research Council has announced the availability of Merck Postdoctoral Fellowships in the Natural Sciences for 1952-53. All fields of chemistry and biology are open to applicants.

The Fellowships carry stipends ranging from $3,000 to $5,000, plus travel expenses, and are offered annually to citizens of the United States with training in chemistry or biology equivalent to that represented by a Ph.D. degree. The program is supported by Merck & Co., manufacturing chemists of Rahway, N. J. Applications must be filed with the Council before January 15, 1952.

Further information and application blanks may be obtained from the Fellowship Office, National Research Council, 2101 Constitution Avenue, Washington 25, D. C.

OBSERVATIONS ON MIDGETS IN BEEF CATTLE*

C. E. LINDLEY†
Washington Agricultural Experiment Station

"BULLDOG" JAW
Figure 25

Midget cattle show extreme prognathism. The under jaw of this heifer was at least an inch longer than the upper jaw.

THE increasing frequency with which "midgets" or "dwarfs" have been occurring in beef cattle makes them of considerable economic importance. One breeder in the State of Washington purchased six bred heifers; four of which produced "midget" calves. These four calves were a total loss to this breeder. Several other breeders have as high as ten to 15 percent of their cows dropping midgets. Few breeders can withstand such high losses.

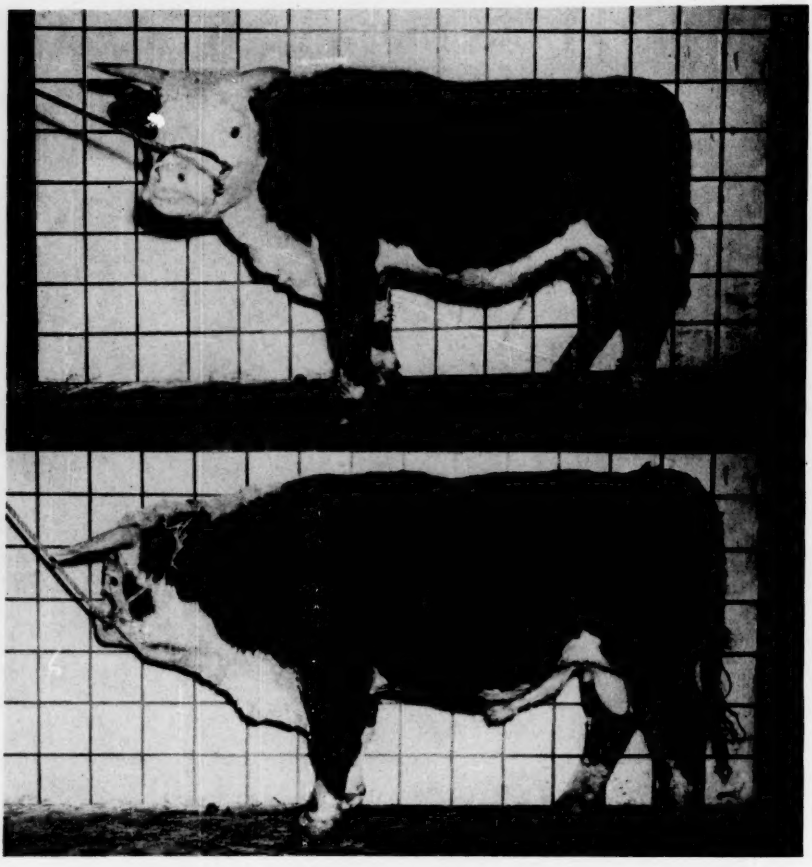
The condition is reported by Johnson *et al.*[3] to be inherited as a monofactorial autosomal recessive in Hereford cattle. Baker *et al.*[1] presented evidence that it is inherited in the same way in Shorthorn cattle. While the author knows of no report of this type of inheritance in Angus cattle, one pure-bred Angus calf, dropped in the Washington State College herd in 1949, weighed 36 pounds at birth, grew very slowly and exhibited a metabolic disturbance similar to that reported for the other breeds.‡

Two Hereford calves, a bull and a heifer, exhibiting conditions very much the same as those described by Johnson

*Scientific Paper No. 1016, Washington Agricultural Experiment Stations, Institute of Agricultural Sciences, The State College of Washington, Pullman.

†Assistant Animal Husbandman. The histological work was done by Dr. G. R. Spencer, Chairman, Department of Veterinary Hygiene and Pathology, College of Veterinary Medicine, State College of Washington, Pullman, Washington.

‡While this article was in press, Baker, Blunn and Plum (JOURNAL OF HEREDITY 42: 141-143, 1951) report the occurrence of nine dwarfs in an Aberdeen-Angus herd. They found no evidence that selection of heterozygotes as breeding stock had been a factor in the appearance of this defect.



MIDGET STIGMATA

Figure 26

Midget Hereford cow and midget Hereford bull two years and nine months of age. Note the typical wide short head, undershot jaw, bulging eyes, crooked hind legs, and extremely small size (the squares in the background are six inches).

et al.[2] were dropped in the Washington State College herd in 1949. The female weighed 47 pounds at birth and was very weak. The male weighed 55 pounds. In addition, the heifer exhibited a decidedly uncoordinated gait and an extremely undershot jaw (Figure 25). They both grew very slowly. The heifer died at about six months of age. While not showing a decidedly uncoordinated gait, the bull calf possessed the other usual

TABLE I.—Weights and measurements on midget and normal cattle.

	Midget bull	Normal bull	Midget cow	Normal cow
Age (month)	33	22	33	33
Weights (pounds)	800.0	1395.0	575.0	1320.0
Height at Withers (in.)	41.0	48.0	38.5	47.0
Height at Hips (in.)	40.0	48.0	36.5	48.0
Length Tail-Head to Shoulder (inches)	40.0	45.0	38.0	53.0
Length of Cannon (in.)	5.0	8.0	4.0	8.0
Length—Eye to Muzzle (in.)	8.0	10.0	7.0	10.5
Length—Poll to Muzzle (in.)	15.5	18.0	14.0	17.0
Width of Head at Eye (in.)	10.0	10.0	8.5	8.5
Dressing Percentage	41.0		41.0	

symptoms. Both calves exhibited bulging eyes. One other calf, very similar to these, was dropped in 1950. It weighed 51 pounds at birth.

In the summer of 1950, two midget Herefords (one cow and one bull) were presented to the Washington State College Animal Husbandry Department. These cattle were just past two years of age at that time. The cow had had one calf removed by Caesarean. The former owner reported that the calf was normal, but no record of its sire is available. One other cow of the same age had died giving birth to a calf. After the cow was brought to Washington State College, she was observed to be in heat on several occasions. At the time of slaughter in late 1950, a fetus was removed from the cow. It is assumed that she was bred by the midget bull, since she was not exposed to any other bull. It is doubtful if she could have supported a normal bull during service.

Figure 26 shows the cow and bull just before slaughter at an approximate age of two years, nine months. It is doubtful if the cow would have lived much longer, since she was becoming extremely weak. Table I presents data on these two midget and normal cattle.

Both midget animals were in very thin flesh even though receiving the same ration as normal cattle. Very little finish was evident at slaughter.

Histological sections were prepared of the pituitary glands, thyroids, adrenals, ovaries, and testicles. The pituitary glands exhibited many large cystic spaces partly filled with a reddish hyalin material. Lucas *et al.*[3] state that the pituitary glands of small type steers may have been under physiological stress. The acini of the thyroids were widely dilated and filled with colloidal material. Lucas *et al.*[3] reported mildly hyperfunctioning thyroids in small type steers. The adrenals and ovaries were apparently normal. One ovary contained a corpus luteum. In one testicle, the seminiferous tubules appeared cellular, but only a few sperm were evident. The other testicle showed hypoplasia of the germinal and interstitial cells.

The similar condition of the thyroid and pituitary glands of small type steers reported by Lucas *et al.*[3] and these "midget" cattle indicate that the two are different degrees of the same condition. If this is true, and if the condition is inherited as reported by Johnson *et al.*[2] and Baker *et al.*[1], the small type steers are possibly heterozygotes as postulated by Johnson[2], and the midgets the homozygotes. This does not satisfactorily explain the case of a 1,365 pound cow, which in no way resembled the small type or midget cattle, producing a midget calf. This cow produced one of the midget calves dropped in the Washington State College herd in 1949. It seems probable that the condition may involve more than one single factor or gene.

Summary

Further observations on the "midget" condition in beef cattle are presented. Birth weights, weights at approximately three years of age and measurements at this age are given. Observations on the histological condition of the testicles, ovaries, pituitaries, thyroids, and adrenals of midget cattle are presented and discussed.

Literature Cited

1. Baker, M. L., C. T. Blunn, and M. M. Oloufa. *Jour. Hered.* 41:243-245. 1950.
2. Johnson, L. E., G. S. Harshfield, and W. McCone. *Jour. Hered.* 41:177-181. 1950.
3. Lucas, K., F. X. Gassner, H. H. Stonaker, and S. S. Wheeler. *Proc. Western Section, Amer. Soc. Animal Prod.* 1950.

(*Received April 24, 1951*)

◆

THE PROBLEM OF EPILEPSY

A Review

IN view of the many loopholes in our current knowledge of the genetics of epilepsy, this meticulously organized and adequately translated report* is another feather in the cap of that Scandinavian branch of medical genetics specializing in the field of clinical psychiatry. It is most welcome. The geneticist will be especially interested in the statistical presentation concerning the frequency of convulsive disorders observed among the parents, siblings and children of a statistically representative sample of 897 epileptic patients. This is not another spirited disquisition on the controversial technical problems arising from the well-

* *A Study of Epilepsy In Its Clinical, Social and Genetic Aspects.* Acta Psychiatrica et Neurologica, Supplementum 63, by Carl Henry Alström (Head of Human Genetics Laboratory No. 2 of the Psychiatric Clinic of the Caroline Institute, Stockholm), Copenhagen, Ejnar Munksgaard, 1950. pp 284.

known complexities of human behavior disorders. Fully in line with the workman-like standards of the Scandinavian school, the emphasis of the investigator's approach has been on the careful procurement of a new series of empirical data regarding the distribution of convulsive phenomena in the families of epileptics.

Possibly because of certain peculiarities in their own training, the reviewers are glad to record at this point that they are somewhat partial to the endeavors of plain and competent workmanship even in research dealing with psychiatric genetics. This partiality has been intensified by the fact that the present era of human genetics in this country (whether post-war or pre-war) seems to be distinguished by a rather deplorable dearth of productive work. It would hardly be profitable to speculate whether this dearth has been caused mainly by the imposing methodological difficulties inherent in this type of investigation or whether, at least in part, it reflects the widespread frustrations of this era, growing out of the chronic anxieties in which our lives are cast because of the unsettled state of the world. It appears unquestionable, however, that in a too-long-neglected segment of research activity such as that pertaining to human inheritance, even the most intriguing ruminations of critical armchair theorists are apt to remain rather valueless, unless they supply the impetus to a repetition of some empirical work which appears to be open to criticism on theoretical grounds.

"The Answer" Still Eludes Us

In the end the author fails to reach his objective of providing a genetically clear and clinically consistent theory of convulsive disease. Nonetheless, his findings are of undeniable interest, especially since they have been obtained by a neurologically trained staff and with the aid of critically applied methods (based on Strömgren's "weight system"). In general, the total mean epilepsy rate for the investigated groups of blood relatives of epileptic index cases (parents, siblings and children) has been found to be remarkably (or perhaps deceptively) low; namely, 1.5 ± 0.18 percent. This rate varies from 1.3 ± 0.27 percent for the parents, to 1.5 ± 0.25 for the siblings, and to 3.0 ± 0.93 for the children. The rate of consanguineous marriages among the parents of the index cases is 2.7 percent for the entire series (without significant variation wtih respect to any particular sub-group). This corresponds rather closely with the general population rate for rural districts in Sweden.

The usual distinction of idiopathic (genetic) and symptomatic (acquired) types of epilepsy has been entirely dispensed with in this study. Instead, an attempt has been made to divide all the index cases included in the investigation into those of known, probably known, and unknown etiology. To no one's surprise, the last group is by far the largest (68 percent). It is surprising that no significant difference in the tendency to convulsive phenomena was observed between the blood relatives of epileptic cases of unknown origin and the blood relatives of those of known or probably known origin.

Approximately one-half of the morbidity risk in convulsive disease has been found to fall into the period below the age of 20 years. After the age of 40, only 20 percent of the total risk remains. An observed deviation in the risk curve of the male (deviating toward a somewhat higher age than that observed in the female) is explained by an increased expectancy of head injuries in men.

From a genetic standpoint, the most relevant conclusion of the author is a negative one. The possibility of a single-factor type of inheritance (dominant or recessive) is rejected not only with respect to a specific convulsive genotype in general, but also with respect to a dominant factor for cerebral dysrhythmia as the basic cause of epilepsy as postulated by Lennox and the Gibbses. In order to support a theory of simple dominance, reduction to less than 10 percent in the penetrance of the postulated morbid genotypes would have to be assumed. The author believes, therefore, that epilepsy is not a specific disease entity based on a "uniform mode of hereditary transmission," but a non-specific clinical syndrome indicating various focal types of "pathological irritation of the central nervous system" as maintained by Penfield.

The eugenic recommendations of the author are moderate, and in conflict with the existing legal restrictions of marriages of Swedish persons afflicted with "hereditary epilepsy." Following a thorough discussion of the medical, social and genealogical aspects of the disease, an emphatic warning is expressed against rigid applications of this restrictive law, especially in persons of satisfactory moral and intellectual standards.

The monograph is richly documented and exceptionally well printed.

B. Aschner and F. J. Kallmann

Department of Medical Genetics, New York State Psychiatric Institute, Columbia University.

GENETIC FOOTNOTES

Fundamental Concepts of Genetics

Dr. E. B. Babcock reviews the seven highlights of the first half-century since Mendel's paper was rediscovered. The bibliography includes the contributions of over a hundred geneticists.

—60c per copy; $4.80 per doz.

Mendel Issue— Journal of Heredity

This issue contains a facsimile reproduction of Mendel's "Experiments in Plant Hybridization" exactly as it appeared in the Verhandlungen Naturforschender Verein in Brunn, Vol. 4, pp. 3-47.

—50c per copy

Meiosis in Maize

Charts and photographs illustrating the different stages of meiosis. 12 pp.

—20c ea.; $1.25 per dozen

Order from:

AMERICAN GENETIC ASSOCIATION
1507 M ST., N.W., WASHINGTON 5, D. C.

PROCEEDINGS of the AMERICAN BREEDERS ASSOCIATION

Vol. I (1904) to combined Vol. 7 & 8 (1911-12)

Each volume in original binding of green cloth. Many of the articles are reports of original research made soon after the rediscovery of Mendel's laws. For reference purposes they have a growing historical interest, and the earlier volumes are very hard to obtain. A few complete sets are still available at $35.00 per set of 7 volumes. Separate copies of Volumes 4-8 also for sale—$4.00 per volume.

◆

1507 M St., N.W. Washington 5, D.C.
American Genetic Association

POPULATION STUDIES

A Journal of Demography

Editor, D. V. Glass

Vol. V, No. 1 **July 1951**

CONTENTS

A. SAUVY. Some Lesser Known French Demographers of the Eighteenth Century: De La Morandière, de Caveirac, Cerfvol and Pinto.

L. T. BADENHORST. Population Distribution and Growth in Africa.

J. W. B. DOUGLAS. Social Class Differences in Health and Survival During the First Two Years of Life; the Results of a National Survey.

COLIN CLARK. Regional Differences in Reproductivity in England and Wales.

F. J. BUCKATZSCH. The Constancy of Local Populations and Migration in England before 1800.

D. V. GLASS. A Note on the Under-Registration of Births in Britain in the Nineteenth Century.

Subscription $5.00 per volume

Published for the POPULATION INVESTIGATION COMMITTEE by

CAMBRIDGE UNIVERSITY PRESS
32 East 57th Street, New York 22

The American Genetic Association

An incorporated organization devoted to promoting a knowledge of the laws of heredity and their application to the improvement of plants, animals, and human racial stocks.

Its membership is composed of men of science, teachers, publicists, physicians, clergymen, parents, students, horticulturists, and breeders of live stock throughout the world.

The Association owns the JOURNAL OF HEREDITY, which is published bi-monthly and sent to each member without additional cost. Every member is thus a part owner of the JOURNAL.

Membership imposes no burdensome obligations. The Association, which is co-operative in nature, welcomes assistance in research, but does not demand it. Members are invited to submit discussions of the results of their research, accompanied by new and unusual photographs. All papers received will be given full consideration by the editorial board.

Manuscripts should be sent to the Editorial Office of the JOURNAL, 1507 M Street, N. W., Washington 5, D. C.

Proof: In order to facilitate prompt publication only galley proof will be sent to authors.

Reprints: On request the author will receive gratis 100 reprints without covers. Additional copies will be furnished at cost.

REQUIREMENTS FOR MEMBERSHIP

Subject to the aproval of the Council, any person interested in the improvment of the human race or the creation of better varieties of plants and animals, is eligible for membership.

The Association welcomes all who are interested in its program, and the Secretary will be glad to answer any inquiries.

Annual dues, giving the right to attend all meetings, and receive the JOURNAL OF HEREDITY, are $4.00 within the United States and its possessions; $4.25 in Canada, and $4.75 in all other foreign countries; life membership, $100. Subscription to the JOURNAL is $5.00 per year (foreign postage extra.)

If you are not already a member, and want to become one, or if you know of anyone who would be interested in membership, write to

THE AMERICAN GENETIC ASSOCIATION

Washington 5, D. C.